51ˢᵗ Annual Symposium / Le 51ᵉ symposium annuel

Optimizing testing strategies and exploring novel mechanisms of human and environmental toxicants

December 2-4, 2019
Shaw Centre
Ottawa, ON

SOCIETY OF TOXICOLOGY OF CANADA
LA SOCIÉTÉ DE TOXICOLOGIE DU CANADA

President: Angela Hofstra, Syngenta Canada Inc.
Vice President: Geraldine Delbes, INRS
Past President: Michael Wade, Health Canada

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ananda
Delivering human models for toxicity testing

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PRESIDENT'S WELCOME

It is our pleasure to welcome you to the 51st Annual STC Symposium. We also take pleasure in thanking our Program Committee who have put together what we hope you will find to be an excellent series of speakers covering themes from alternatives to traditional toxicity tests to long term and trans-generational impacts of toxicant exposure. We are very grateful for the support of our sponsors without whom we would not be here. We also thank the many of you who have chosen to present their work at our meeting and note the breadth and excellence of abstracts – particularly from the trainees - to be presented in our poster sessions. Judging from these, the future of our discipline looks bright. Here's hoping that you have a satisfying and productive time in Ottawa and we look forward to discussing toxicology with you all!

Geraldine Delbes, Angela Hofstra, Michael Wade

Vice-President, President and Past President Respectively.

LOCATION OF ON-SITE EVENTS

Symposium will be held at the **Shaw Centre**

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Posters and Exhibits/Vendors	215
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Lunch December 3,4	213

Shaw Centre – Level 2
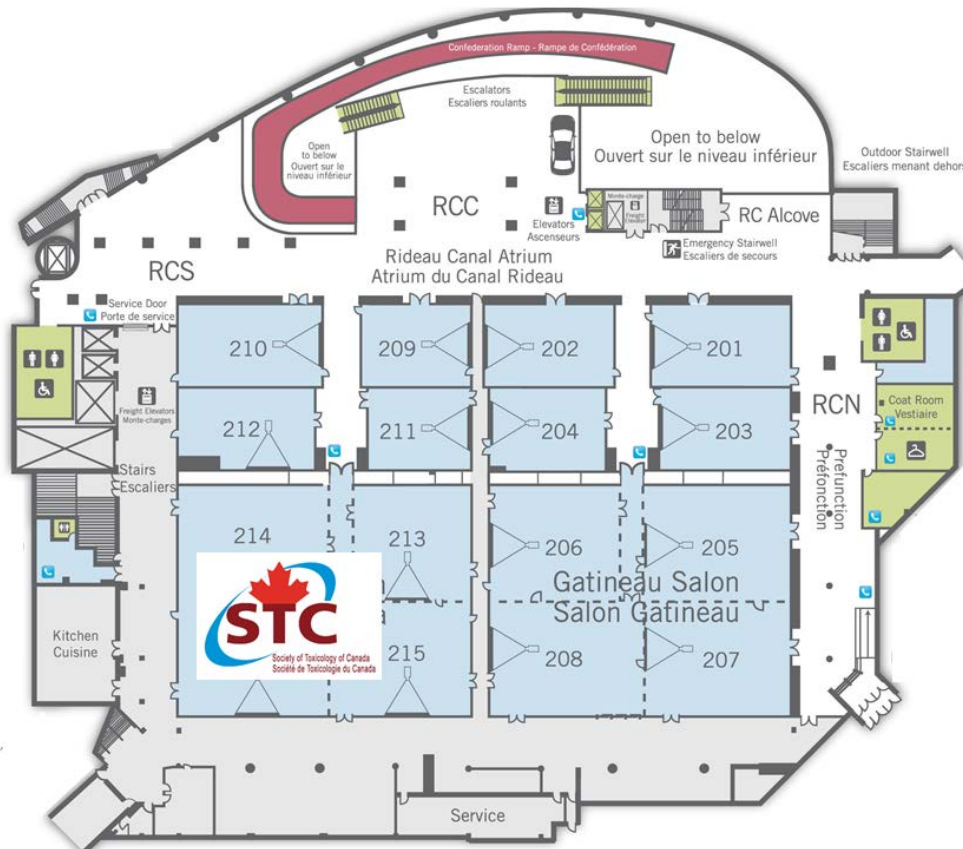
LOCATION OF RECEPTIONS

Welcome Reception:

Aulde Dubliner & Pour House

62 William St.,

Byward Market

613 241-0066

Shaw Centre

Les Suites Hotel

President's Reception

240 Sparks St.

www.les3brasseurs.ca

+1 613-380-8140

3 Brewers Pub

Shaw Centre

SYMPOSIUM PROGRAM

Monday December 2
7:00 pm   Welcome Reception: The Pour House, 62 William St.

Tuesday December 3
7:30 am   Registration Shaw Centre

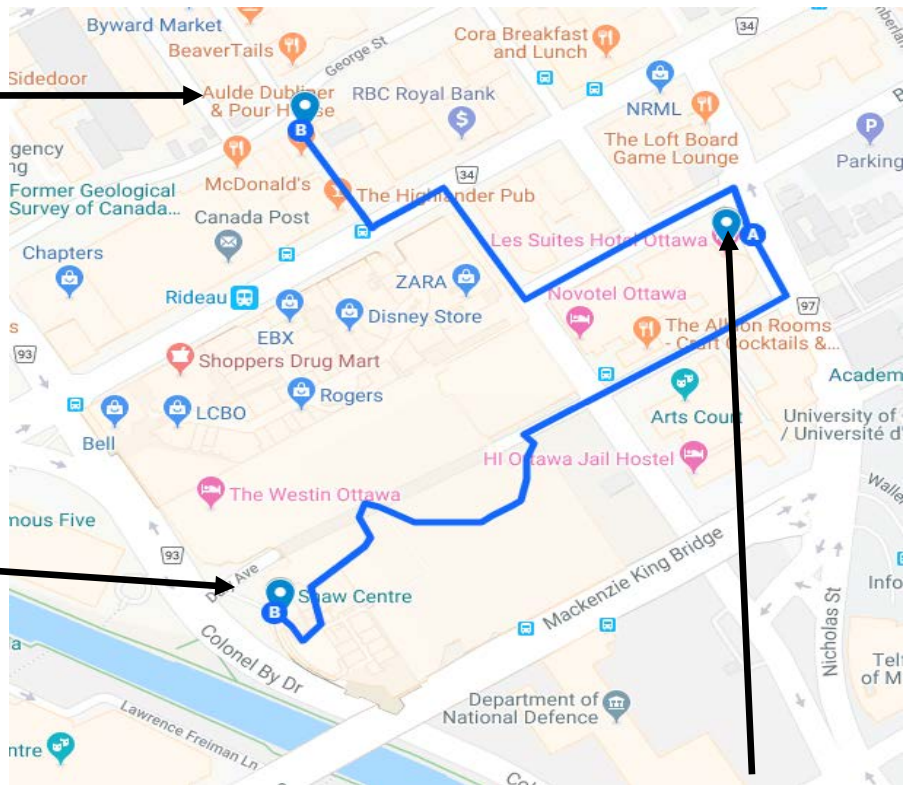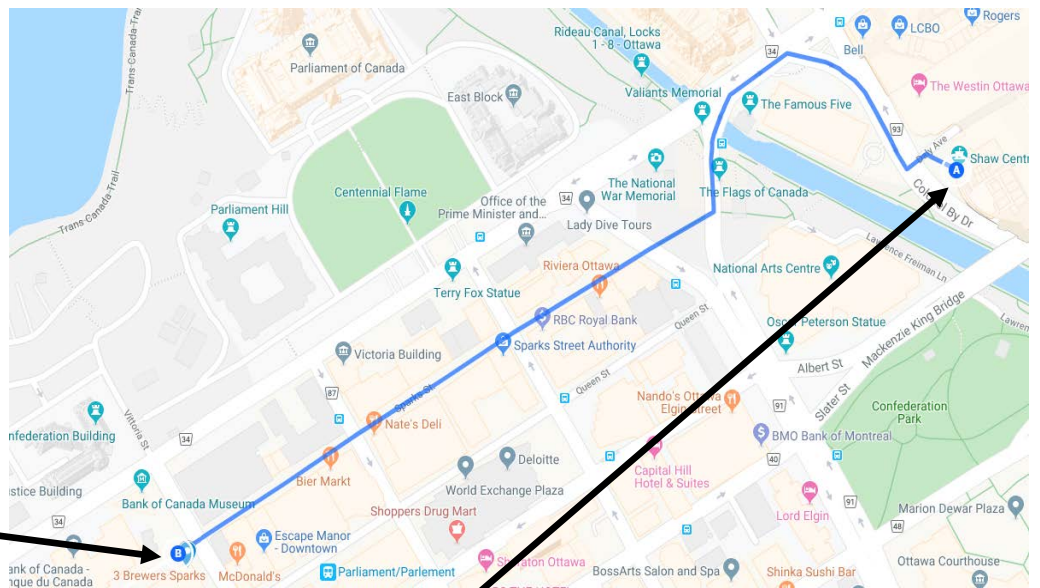**8:50 am Welcome and Acknowledgment of the Land:** Angela Hofstra, STC
President (Syngenta Canada, Guelph, ON)

9:00 am   Session 1: Alternative models for predicting human toxicity.
Chair: Joanne Wan, Intertek

9:00 - 9:30   Michael (Rocky) Goldsmith (Lead Computational Discovery Chemistry, Bayer Crop
                Science, USA): Molecular De-risking by Design: Modeling and Simulation
                to Advance Safer Chemistries.

9:30 - 10:00   Margaret Magdesian (CEO Ananda Devices, Montréal, Canada):
                Advantages and challenges of human models on-a-chip.

10:30-11:00 Coffee break and poster viewing

11:00 - 11:30 Kessen Patten (Institut National de la Recherche Scientifique,
                Montréal, Canada): Zebrafish: An animal model for toxicological studies.

11:30 - 12:00 Alisa Vespa (Health Canada): Assessing the Human Cancer Risk of
                Pharmaceuticals: Potential changes to the use of the 2-year
                rat carcinogenicity study.

12 - 1:15 Lunch and poster viewing

**1:15 pm Session 2: Transgenerational Effects – "You are what your grandfather
ate"**
Chair: Mike Wade, Health Canada

1:15 -1:45   Janice Bailey (Laval University, Canada): Molecular Foundations of
                Multigenerational Transmission of the Paternal Environment.

1:45 - 2:15   Francesco Marchetti (Health Canada) Advances in assessing the effects
                of lifestyle and environmental exposures on paternally transmitted genetic
                damage.

2:15 -2:45   Vance Trudeau (University of Ottawa): The antidepressant Prozac is a transgenerational neuroendocrine disruptor of behaviour and stress in a fish model: is it time to raise the warning flag for human health?

2:45 – 3:15 Coffee Break and Poster viewing

3:15 – 4:00 Keynote Lecture: Jodi Flaws (University of Illinois at Urbana-Champaign, USA) The effects of phthalates on female reproduction.

4:00- 5:30 Student Mentoring Event & STC Annual Business Meeting
Theme: Take the Initiatives!
Co-Chairs: Yen Tran, Carleton University (Ottawa ON) & Lorrie Boisvert, University of Ottawa (Ottawa ON)

Mentor panellists:
Andrew Beck, Director, Risk Management Bureau, Health Canada (Ottawa ON)

Dr. Moazzam Khan, Senior Toxicologist, Existing Substances Risk Assessment Bureau, Health Canada (Ottawa ON)

Dr. Laurie Chan, Professor and Canada Research Chair in Toxicology and Environmental Health, Department of Biology, University of Ottawa (Ottawa ON)

Dr. Leanne Bedard, Principle and Consultant, Bedard ADME-Tox Solutions (Montréal QC)

6:00 pm President's Reception and STC Awards: 3 Brewers Pub, 240 Sparks St.

Wednesday December 4

8:00 am Registration

8:30 am Session 3: Thinking outside the reproductive organs: novel toxicity mechanisms of EDCs.
Chair: Ella Atlas, Health Canada

8:30 – 9:00 Jenny Bruin (Carleton University, Ottawa, Canada): Dioxin effects on human primary pancreatic beta cells generated from stem cells.

9:00 – 9:30 Vian Peshdary (Health Canada): Dechlorane Plus inhibits insulin signalling and causes adipose tissue dysfunction in mice.

9:30 – 10:00 Errol Thomson (Health Canada): Role of the stress axis in mediating health impacts of air pollutants.

10:00 – 10:30 Coffee break and poster viewing

Session 4: Plaa Award Presentation and Lecture.
Chair: Angela Hofstra, President

10:30 – 11: 15 Jack Uetrecht (University of Toronto):The Promise and Perils of Immunotoxicology.

11:15 – 12:15 Session 5: Invited Trainee (Students/Postdoctoral Fellows) Platform Presentations.
Chair: Isabelle Plante, INRS-IAF, Laval

11:15 – 11:30 Elyse Caron-Beaudoin - Is there an association between proximity and density to hydraulic fracturing wells and birth outcomes?: a study in Northeastern British Columbia, Canada.

11:30 – 11:45 Mercedes Rose - Air Pollution and the Stress Axis: Regulation of Tryptophan Metabolism, Serotonin Receptor and Neurotrophic Factor Expression.

11:45 – 12:00 Laetitia Lecante - Acetaminophen (APAP) may interfere with the human fetal ovary development in an ex vivo model.

12:00 – 12:15 Anthony Reardon - Application of high-throughput transcriptomics in human liver spheroids to facilitate read-across data for risk assessment of 24 per- and polyfluoroalkyl substances (PFAS).

12:15 – 1:15 Lunch and poster takedown

1:15 – 3:00 Session 6: Assessment of effects of environmental pollutants on humans & other species
Chair: Geraldine Delbes, INRS - IAF, Laval

1:15 – 1:45 Valérie Langlois (Institut National de la Recherche Scientifique; Montréal, Canada): Effects of the pipeline transported diluted bitumen in the non-mammalian embryos.

1:45 – 2:15 Doug Crump (Environment and Climate Change Canada): Application of new approach methods for evaluating the effects of priority chemicals and complex mixtures in avian species.

2:15 – 2:45 Beverley Hale (University of Guelph, Canada): Bioaccessibility estimates by gastric SBRC method to determine relationships to bioavailability of nickel in ultramafic soils.

2:45 – 3:00 Closing Remarks Angela Hofstra, STC President (Syngenta Canada, Guelph, ON)

BIOSKETCHES: SPEAKERS

Dr. Jodi A. Flaws is a Professor in Comparative Biosciences at the University of Illinois-Urbana/Champaign. She received a B.S. in Biology from St. Xavier University, a M.S. in Biology from Loyola University of Chicago, and a Ph.D. in Physiology from the University of Arizona. Following completion of the Ph.D. degree, Dr. Flaws performed postdoctoral research at Johns Hopkins University and the University of Maryland. Following postdoctoral training, Dr. Flaws accepted an Assistant Professor position at the University of Maryland, where she subsequently was promoted to Associate Professor. In 2006, Dr. Flaws accepted a position as Professor of Comparative Biosciences at the University of Illinois-Urbana/Champaign. Dr. Flaws' research program is mainly focused on determining the mechanisms by which environmental chemicals affect the development and function of the ovary. Her research is funded by grants from the National Institutes of Health and Environmental Protection Agency. She has published over 200 peer-reviewed papers that have involved extensive participation and authorship by graduate students, postdoctoral fellows, veterinary medical students, and undergraduate students. She is the recipient of the Department of Epidemiology and Preventive Medicine, University of Maryland Student Mentoring Award, the Patricia Sokolove Outstanding Mentor Award, the Dr. Gordon and Mrs. Helen Kruger Research Excellence Award, the Pfizer Animal Health Award for Research Excellence, the University Scholar Award, the Women in Toxicology Mentoring Award from the Society of Toxicology, and the Society for the Study of Reproduction Trainee Mentor Award.Jodi Flaws

Dr. Michael ("Rocky") Goldsmith is the Computational Discovery Chemistry Lead for theNovel Modalities Platform of Small Molecules Research Emerging Technology at BayerCrop Sciences in Chesterfield MO. Prior to this position he was at Chemical ComputingGroup Inc., a Montréal-based life-science informatics and modeling software companythat produces the Molecular Operating Environment (MOE); a fully integrated molecular-discovery platform. Dr Goldsmith also worked for nearly a decade as a principalinvestigator at the US EPA, where he also performed post-doctoral research at theNational Center for Computational Toxicology in the field of in silico chemical genomicsmethods and virtual high-throughput screening, and later started much of thecomputational exposure sciences with a focus on Consumer Product chemicalexposures. During his undergraduate studies at Concordia University in Montréal heworked in pharmaceutical, tobacco and explosives industries in both R&D and productdevelopment and subsequently completed his Ph.D. in theoretical chemistry andmolecular biophysics at Duke University (Durham, North Carolina) working on theoretical optical activity of molecular assemblies and aggregates, theory-assisted determination of absolute stereochemistry of chiral natural products, and elucidating the molecular mechanisms of biological sequestration and in vivo distribution of the food contaminant Ochratoxin.

Dr. Margaret Magdesian, BSc Pharmacology, PhD Biochemistry, is a scientist-entrepreneur with over 20 years of experience in biopharmaceutical research. In collaboration with researchers from McGill University, Dr Magdesian has developed innovative technology to rapidly grow human nervous-system-on-a-chip. In 2015 she launched the company Ananda Devices to commercialize this technology and to enable pharma, food and cosmetic companies to perform toxicity testing, drug screening, drug repurposing and disease modelling up to 50x faster and 90% more cost-effective than current methods. Ananda Devices is a certified Women Owned SME, based in Montréal, that help companies comply with legislations to reduce animal experimentation by supplying platforms of human tissues-on-a-chip. As CEO of Ananda Devices, Dr Magdesian won over 25 grants and awards in science and innovation including the 2016 Top 10 Quebec Discovery of the Year, the 2018 Top 7 SheEO in Canada, 2018 Canadian Export Challenge and 2019 Cartier Women's Initiative Awards.

Dr. Kessen Patten is an Assistant Professor at the INRS-Centre Armand Frappier Santé et Biotechnologie in Quebec, Canada. He did his PhD in Physiology and Cell Biology at the University of Alberta, followed by two postdoctoral trainings at the Université de Montréal. During his academic training, Dr. Patten has provided new insights in mechanisms of synaptic development using zebrafish, discovered a new gene associated with idiopathic scoliosis and identified neuroleptics as potential therapeutics for amyotrophic lateral sclerosis (ALS), with one compound currently in clinical trial.

The Patten lab uses the zebrafish to model developmental genetic disorders and neurodegenerative diseases such as ALS for new genetic identification and drug discovery. His lab has also recently started to exploit the advantages of the zebrafish for toxicological studies. Dr. Patten is the recipient of the ALS Canada and Brain Canada Career transition award as well as a Fonds de recherche du Québec – Santé (FRQS) Junior 1 Research Scholar.

Dr. Alisa Vespa joined Health Canada in 2006. She worked several years as a Senior Evaluator in the Bureau of Metabolism, Oncology and Reproductive Sciences evaluating non-clinical data for new drug submissions. More recently, Alisa joined the Office of Risk Management to work on the issue related to nitrosamine impurities in pharmaceutical products. Alisa is also Health Canada's topic leader for the ICH S1 and M7 expert working groups and deputy topic leader for the ICH Q3C and Q3D expert working groups. Alisa holds a B.Sc. in Physiology from McGill University and a Ph.D. in Pharmacology and Toxicology from Western University.

Dr. Janice Bailey is the Scientific Director of the Fonds de recherche du Québec - Nature and Technologies (FRQNT) since March 2019. Prior to joining the FRQNT, she was a Professor of Animal Sciences & Research Associate Dean, Faculty of Agriculture & Food Sciences, Adjunct Professor in the Faculty of Medicine, Laval University
(Québec City, Canada). Her research explores the impact of the environment, such as toxicant exposure and nutrition, on reproductive development and the ability to produce healthy offspring across multiple generations. She is particularly interested on paternally-mediated developmental outcomes, which has been relatively ignored. She is

a founding member of the Centre de recherche en reproduction, développement et santé intergénérationnelle, composed of researchers from the Faculties of Medicine and Food & Agricultural Sciences. Professor Bailey has served as a member and chair of grant review panels in Canada and the USA, and on the editorial boards of several journals. She has been involved in the organisation of numerous international congresses in Canada, the USA, Australia, Denmark and South Africa and has been elected to executive governance roles in various national and international scholarly societies

Dr. Francesco Marchetti is a research scientist at Health Canada and an internationally recognized expert in germ cell mutagenesis. His research focuses on the identification and characterization of environmental mutagens that affect the genetic integrity of germ cells, the transmission of genetic damage to the offspring and its health consequences. Dr. Marchetti is an author on over 110 peer-reviewed publications. He is an Adjunct Research Professor at Carleton University in Ottawa, the chair of the Germ Cell Workgroup of the HESI's Genetic Toxicology Technical Committee and serves on the expert group on genetic toxicology for the OECD.

Dr. Vance Trudeau has a long record of accomplishments in the area of fish and amphibian endocrinology and ecotoxicology. With more than 270 publications, Trudeau's research has been impactful in many ways, but especially related to hormonal control of reproduction, and how it is disrupted by common pollutants, including human pharmaceuticals, pesticides, and petroleum-derived contaminants. His current work is leading to new discoveries on neurotransmitters and neuropeptides, and how the brain itself can make sex hormones (e.g., estrogens). Trudeau is known worldwide for his work on environmental pollutants and how they can disrupt brain pathways, leading to suppression of sexual function. In the latest research from him team, he has shown how fluoxetine, the active ingredient in the antidepressant Prozac, can have persistent transgenerational effects in fish, which will be the subject of his presentation.

Dr. Jenny Bruin is an Assistant Professor at Carleton University in the Department of Biology and Institute of Biochemistry, since September 2016. Her lab studies the pathogenesis of diabetes with a focus on islet biology, pancreas development, and toxicology. From 2010 to 2016, Dr. Bruin was a postdoctoral fellow in Dr Tim Kieffer's laboratory at the University of British Columbia, where she studied the development of human embryonic stem cells into pancreatic insulin-producing beta cells as a potential cell therapy for patients with diabetes. Dr Bruin obtained her BSc in Biomedical Toxicology at the University of Guelph in 2005 and her PhD in Medical Sciences with Dr. Alison Holloway at McMaster University in 2009.

Dr. Vian Peshdary is a postdoctoral scholar at Case Western Reserve University (Cleveland, Ohio) in the Department of Genetics, since this past October (2019). Previously Vian was a postdoctoral fellow in Dr. Ella Atlas' lab at Health Canada studying the effects of endocrine disrupting chemicals on adipogenesis, insulin resistance and metabolic syndrome from 2016-2019. Vian completed her doctoral training at Ottawa Hospital Research Institute under the supervision of Dr.

Alexander Sorisky. Her doctorate thesis was assessing the effects of high glucose – mediated macrophage secreted factors on adipogenesis and adipocyte biology.

Dr. Errol Thomson is a Research Scientist in the Environmental Health Science and Research Bureau of Health Canada. He has over 15 years' experience studying mechanisms underlying health impacts of air pollutants. On the basis of his work showing that air pollutants activate neuroendocrine stress response systems, his research now explores the relationship between environmental exposures, individual sensitivity, and health.

Dr. Valérie Langlois is an Associate Professor at the Institut national de la recherche scientifique (INRS) located in Quebec City (QC) and holds a Canada Research Chair in Ecotoxicogenomics and Endocrine Disruption. She is an animal physiologist and ecotoxicologist who specialized in the characterization of the effects and the molecular mechanisms of action of environmental contaminants in non-mammalian species. Dr. Langlois mostly uses an 'omics approach to address her research questions and she has long worked with contaminants that are known to be endocrine disruptors. Her research lab currently encompassed 2 post-doctoral fellows, 7 Ph. D., 2 M. Sc., and 2 research associates.

Doug Crump is a Research Biologist with Environment and Climate Change Canada. His research laboratory at the National Wildlife Research Centre evaluates the potential applications of 'omics technologies, in vitro methods and other alternative testing strategies (e.g. early-life stage exposures) to determine the effects of priority environmental chemicals and complex mixtures in avian species. Mr. Crump is also involved with an extensive Great Lakes field program through which wildlife samples are collected from variably contaminated areas to evaluate cumulative effects in natural populations.

Dr. Beverley Hale was appointed as the Associate Vice President Research (Agri-Food Partnership) at the University of Guelph in January 2018, where she manages the agreement between OMAFRA and the University of Guelph. With an annual funding flow of about $100M, this agreement supports faculty, graduate students, research operating costs, field research stations and two analytical laboratories, in their pursuit of agri-food research and innovation. Dr. Hale is also a Professor in the School of Environmental Sciences, where her research program (toxicological risk assessment of metal-contaminated soils) is a strong applied research enquiry that partners with industry and government to deliver the needs of civil society, which is underpinned by discovery-based research, and is substantially funded by the Tri-Council. She has authored or co-authored 79 publications in peer-reviewed journals, has 3369 citations, and 27 completed graduate theses. Her career total research funding is more than $9M including the awarding of an NSERC Strategic Network "Metals In The Human Environment" (2005-2009). In 2011, she was awarded $1M by Environment Canada to create leveraged research collaborations to fill data gaps in support of their policies around the Chemicals Management Plan (CMP) for inorganic contaminants; this Contribution Agreement was renewed in 2017 for a further five years.

BIOSKETCHES: MENTOR PANELISTS

Andrew Beck obtained his B.Sc. in Biology and Environmental Sciences from Trent University in Peterborough, ON, and a Graduate Diploma in Ecotoxicology from Concordia University in Montréal, QC. Andrew began his public service career in 1999 with the New Substances Program at Health Canada: he held numerous positions within the organization including Biologist, Section Head, and Acting Director. During this time Andrew gained extensive experience in exposure, hazard and risk assessment of new chemicals, polymers, products of biotechnology, nanotechnology and the environmental assessment of food and drug substances. In 2012, Andrew joined the Hazardous Materials Information Review Commission (HMIRC) as Director of the MSDS Compliance Division. He led the effort to integrate the independent commission into the Health Canada Portfolio and to introduce the implementation of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). In 2015, Andrew was appointed Director of the Risk Management Bureau (RMB) within the Safe Environments Directorate of Health Canada whose mandate is to promote and protect the health of Canadians by developing, implementing and communicating strategies to manage risks to human health associated with exposure to existing substances in use in Canada.

Dr. Leanne Bedard obtained her M.Sc. in Chemistry from Carleton University in Ottawa, ON, in 1999, and her PhD in Pharmacology and Toxicology at Queen's University in Kingston, ON, in 2004. Leanne's career began in 2006 in drug research and development at Merck Frosst in Montréal as a Senior Research Scientist in Preclinical Drug Metabolism and Pharmacokinetics (DMPK). She advanced several drug discovery projects for respiratory and infectious diseases; her work reduced late-stage attrition of drug candidates due to poor DMPK properties. In 2010, Leanne received the Special Achievement Award for her team's discovery of PIFELTRO™/DELSTRIGO™, a non-nucleoside reverse transcriptase inhibitor now marketed for HIV/AIDS treatment. In 2011, Leanne joined AstraZeneca R&D Montréal as DMPK Drug Design Leader; she worked closely with drug discovery teams to solve ADME-Tox issues in lead compounds and series for the treatment of central nervous system (CNS) disorders. Since 2012, Leanne worked as an independent consultant specializing in drug metabolism, pharmacokinetics, and nonclinical toxicology. Leanne assisted client companies in problem-solving of ADME-Tox issues and in the design and data interpretation of nonclinical pharmacokinetic and Investigational New Drug (IND)-enabling toxicology studies. Leanne worked with over 40 biotech and small pharmaceutical companies in the US and in Canada. She supported the discovery, nonclinical development and successful regulatory filing for several small molecule drug candidates in jurisdictions worldwide and across multiple therapeutic areas including oncology, infectious diseases, inflammatory diseases, pain, hematological and CNS disorders. Leanne currently holds board certifications granted by the American Board of Toxicology (DABT) and the Regulatory Affairs Professionals Society (RAC).

Dr. Laurie Chan was born in Hong Kong and obtained his B.Sc. and M.Phil. at the University of Hong Kong, and his Ph.D. in Toxicology at the University of London. Previously, he held faculty positions at McGill University and the University of Northern British Columbia before he joined the University of Ottawa as Professor and Canada Research Chair (Tier 1) in Toxicology and Environmental Health. For more than 25 years, Dr. Chan has been a world-renowned expert in mercury toxicology and has worked with Indigenous populations. His research in environmental and nutritional toxicology spans from the lab developing new techniques for contaminant analysis, to participatory research in the community on the risk and benefits of traditional foods and impact of environmental change on food security. He has published over 200 peer-reviewed scientific papers and supervised over 80 graduate students. Furthermore, Dr. Chan has also served as an advisor for international and national governments and organizations and numerous Indigenous communities on environmental health issues. He is recognized as a Fulbright Scholar and a Fellow of the Canadian Academy of Health Sciences.

Dr. Moazzam Khan experienced highly diverse research training at prestigious institutions in the United States and Canada. His training includes a Doctor of Veterinary Medicine (DVM), MSc in Veterinary Pathology, MS in Veterinary Medical Sciences, and a PhD degree in Toxicology. Through these experiences, he developed expertise in endocrine disruption, toxicogenomics, veterinary medical sciences, pathology, and chemical risk assessment. After his graduate degree program from the University of Illinois at Urbana-Champaign, USA, Dr. Khan completed post-doctoral trainings at the United States Environmental Protection Agency (US EPA) and Health Canada (HC). Over the past 10 years, Dr. Khan has been working as Senior Evaluation Officer (Toxicologist) at Health Canada, where he conducts risk assessment of chemicals prioritised under the Canadian government's mandate. He has provided expert advice to colleagues and senior management, and engaged with various stakeholders from government, academia and industry. He has mentored staff and many co-op students, as well as participated in the examination or interview of potential scientific staff. Throughout his career, Dr. Khan has led many projects, published research work and won several international fellowships.

POSTER ABSTRACT LIST

Note: Please mount your posters on the board bearing your poster number using the fasteners provided. Posters should be mounted prior to 8:30 am on Dec 3 and removed before 1:15 on Dec 4.

1 - Arsenic is a unique modulator of macrophage polarization toward pro-atherogenic phenotypes
Kiran Makhani

2 - Identifying target organs and candidate contaminants based on adverse outcomes following sub-chronic oral exposure in rats to contaminated soil extracts from a pesticide manufacturing site.
Bright Boamah

3 - Gestational exposure to valproic acid upregulates total Stat3 protein expression while downregulating phosphorylated Stat3 in CD-1 mouse embryos with neural tube defects
Sidra Shafique

4 - Is there an association between proximity and density to hydraulic fracturing wells and birth outcomes?: a study in Northeastern British Columbia, Canada
Elyse Caron-Beaudoin

5 - Consequences of in utero benzene exposure on fetal DNA repair and topoisomerase IIα in CD-1 mice
Trent Holmes

6 - Vulnerability of the maternal brain: Effects of gestational and postpartum exposure to an ecologically relevant toxicant mixture
Anne TM Konkle

7 - Toxicity of Aryl Phosphate Flame Retardants Mediated by Serine Hydrolase Inhibition in Rat Liver, Ovary and Adrenal Glands
Michael Wade

8 - Towards routine detection of endocrine disrupting chemicals in complex effluents in Quebec
Valérie Langlois

9 - Screening for the safety of emerging plasticizers and flame-retardants
Sarah Tardif

10 - Investigating Whether Epigenetic Changes in Murine Fetal Livers Occur Following Gestational Exposure to the Flame Retardant Triphenyl Phosphate
Sydney Wolpert

11 - Investigating the effects of in utero benzene exposure on murine fetal gene expression of Xrcc5
Christian Bile

12 - Using in vitro polybrominated diphenyl ether (PBDE) neurotoxicity data to establish acceptable exposure levels for developing children
Sherri Bloch

13 - Acetaminophen (APAP) may interfere with the human fetal ovary development in an ex vivo model.
L. Lecante

14 - The role of endothelial-targeted PPARγ in mammary tumourigenesis and mammary tumour angiogenesis.
Shi JY

15 - Human Red Blood Cell Uptake and Sequestration of Arsenite and Selenite: Evidence of Seleno-bis(S-glutathionyl) Arsinium Ion Formation in Human Cells
Elaine M Leslie

16 - Effects of environmentally-relevant mixtures of organophosphate ester (OPE) flame retardants on KGN cells, a human granulosa cell line
Xiaotong Wang

17 - An environmentally relevant mixture of organophosphate ester flame retardants negatively impacts endochondral ossification
Han (Aileen) Yan

18 - Tungsten Alters DNA Damage Repair Mechanisms
Rowa Bakadlag

19 - Increase of the adult mammary gland weight after in utero and lactational exposure to Di(2-ethylhexyl) phthalate (DEHP): a complex story
Bélinda Crobeddu

20 - Potential role for endocrine disrupting chemicals in the etiology of postpartum depression
Alexandra Ogilvie

21 - Early immune changes induced by clozapine: potential insights into the mechanism of idiosyncratic drug-induced agranulocytosis
Samantha Sernoskie

22 - In utero and lactational exposure to an environmentally relevant mixture of brominated flame retardants induces precocious development of the mammary glands
Rita-Josiane Gouesse

23 - Predicting the placental transfer of chemicals using quantitative structure-activity relationship (QSAR) modeling
Laura Lévêque

24 - Air Pollution and the Stress Axis: Regulation of Tryptophan Metabolism, Serotonin Receptor and Neurotrophic Factor Expression
Mercedes Rose

25 - DNA methylation dynamic in rat male germ cell during gametogenesis and spermatogenesis
Rhizlane El omri

26 - Optimization of a 3D co-culture model and bilayered co-cultured model as biologically representative alternatives for mammary gland toxicology.
Melany Juárez

27 – Binding Partners of Glutathione Transferase GSTT1
Amy Hoff

28 - Cyanonitroanilines (Aminonitrobenzonitriles): Genotoxicity Studies; Characterization of a Novel Cyclic Deoxyguanosine Adduct
P. David Josephy

29 - Neutrophilia in immune checkpoint blockade mouse model of idiosyncratic carbamazepine-induced liver injury
Alison Jee

30 - Characterizing the low dose effects of methylmercury in early developmental stage using cultured human embryonic stem cells
Bai Li

31 - Impact of copper oxide particle solubility on lung epithelial cell toxicity: response characterization using global transcriptional analysis
Andrey Boyadzhiev

32 - Covalent binding of trimethoprim to liver and epidermal skin proteins
Yanshan Cao

33 - Dimethoxymethane: A Case Study in Applying the Hazard-based Approach to Inhalation-based Exposures in a Screening Assessment within the Chemicals Management Plan
Nazem El Husseini

34 - Identifying transcriptional changes in early placentation-related pathways following exposure to naphthenic acids
Laiba Jamshed

35 - Quantitative structure-activity relationship (QSAR) modeling as a tool to assess lactational exposure for data-poor chemicals
Nadia Tahiri

36 - Does exposure to dibenzothiophene and its alkylated analogue, 2,4,7-trimethyldibenzothiophene, affect key pathways important for placental trophoblast cell function?
Sergio Raez-Villanueva

37 - Effect of sulphur-containing heterocyclic aromatic hydrocarbon exposure on pancreatic beta cell health
Ineli Perera

38 - Approaches for Pesticide Residue Analysis in Cannabis Products for the Canadian Market – Instrumentation and Methods
Richard Bagshaw

39 - Characterisation of the epigenetic reprogramming in perinatal male rat germ cells and its sensitivity to ethinylestradiol
Arlette Rwigemera

40 - Sub-acute and -chronic toxicological responses in male and female F344 rats following repeated oral dietary exposure to the processing-induced food contaminant 2-monochloro-1,3-propanediol
Jayadev Raju

41 - A population-based study to explore the association between environmental contaminants and relevant cancers in Newfoundland and Labrador
Md Arifur Rahman

42 - The Effects of BPA and its Analogues on Aipocyte Differentiation
Misha Singh

43 - Study of the metal content of species of interest for fishing (demersal and pelagic) of the Galapagos Islands. Risk evaluation
Carmen Rubio Armendariz

44 - Trace elements and reproductive success of river otters (Lontra canadensis) in the Alberta Oil Sands Region
Robert Gutgesell

45 - Naphthenic acids and metabolic health: a focus on ANGPTL4
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46 - Application of high-throughput transcriptomics in human liver spheroids to facilitate read-across data for risk assessment of 24 per- and polyfluoroalkyl substances (PFAS)
Anthony J.F. Reardon

47 - Examining the predictors of circulating arsenic levels in the Canadian population
Katherine Pullella

48 - Impact of anthocyanin-rich meals on polychlorinated biphenyl-induced intestinal dysbiosis, oxidative stress and inflammation
Fang Lu

49 - Development of DNA methylation based in vitro assay for the identification of carcinogens
Daniel Desaulniers

50 - Development and application of an analytical pipeline for high-throughput gene expression profiling of per- and poly-fluoroalkyl substances (PFAS) in primary liver human spheroids to inform read-across
Andrea Rowan-Carroll

51 - Synthesis and Characterization of a Novel C8, N7-Deoxyguanosine Cyclic Adduct
Trevor W. Manning

52 - Variability in stress axis function alters ozone-induced lung immune cell responses
Jith Thomas

53 - In Vitro Determination of Drug Transporter Interactions Using LC-MS/MS Quantitation
Albert Licollari

54 - Cytotoxicity screening and ultrastructural study of nonporous silica nanoparticles uptake by mammalian cells
Dalibor Breznan

55 - Nano silica exposure and protein changes in mitochondria from J774 macrophage cells
Prem Kumarathasan

56 - Docosahexaenoic acid (DHA) derived oxylipins are decreased in the heart by dietary exposure to 2-monochloro-1,3-propanediol
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POSTER ABSTRACTS

1 - Arsenic is a unique modulator of macrophage polarization toward pro-atherogenic phenotypes

Kiran Makhani[1], **Nicolas de Jay**[2], **Coralie Charpy**[4], **Luis Fernando Negro Silva**[1], **Maryse Lemaire**[4], **Claudia Kleinman**[2], **Koren K. Mann**[1,3]

[1]*Division of Experimental Medicine, McGill University, Montréal, QC, Canada,* [2]*Department of Human Genetics, McGill University, Montréal, QC, Canada,* [3]*Department of* Oncology, *McGill University, Montréal, QC, Canada,* [4]*Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, QC, Canada*

Arsenic toxicity is correlated with atherosclerosis. Atherosclerotic plaque formation is a complex process with macrophages being major players in both its initiation and progression. They accumulate lipids and modulate the microenvironment through secreting cytokines and recruiting other immune cells to the site. Here, we hypothesized that arsenic exerts proatherogenic effects by skewing the relative abundance plaque-resident macrophages towards a pro-inflammatory M1 phenotype. To test this, we cultured bone marrow derived macrophages from C57BL/6 mice *in vitro* and differentiated them into M1 (IFNg) or M2 (IL-4) phenotypes in the presence or absence of 50 ppb arsenic over 48 hours. Gene expression was assessed by RNA sequencing. The principle component analysis showed that arsenic altered different gene expression within each subtype (namely, M0, M1, M2). Within M2 macrophages, CCL17 and CCL22 mRNA and their secreted protein levels were decreased in the presence of arsenic. These anti-inflammatory chemokines recruit Tregs leading to the plaque resolution. We will correlate this finding to decreased Tregs and increased plaque size *in vivo*.

2 - Identifying target organs and candidate contaminants based on adverse outcomes following sub-chronic oral exposure in rats to contaminated soil extracts from a pesticide manufacturing site.

Bright Boamah[1], **Sarah Barnsley**[1], **Lian Finch**[1], **Jennifer Briens**[1], **Steven Siciliano**[1], **Natacha Hogan**[1], **Markus Hecker**[1], **Mark Hanson**[2], **Patrick Campbell**[3], **Kris Bradshaw**[4], **Aditya Manek**[5], **Ahmad N Al-Dissi**[6], **Lynn Weber**[1]

[1]*Toxicology Centre, University of Saskachewan, Saskatoon, SK*, [2]*Environment and Geography, university of manitoba, Winnipeg, MB*, [3]*Wood Environment & Infrastructure Solutions, Winnipeg, MB*, [4]*Federated Co-operatives Limited, Saskatoon, SK*, [5]*College of Medicine, University of Saskatchewan, Saskatoon, SK*, [6]*Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK*

Soil sample extracts from a legacy contaminated site were examined using an effects-directed approach. The study aimed to identify target organ toxicities after twice-weekly oral gavage of 3 different soil extracts (vehicle control and 0.1% of each extract in polyethylene glycol) in male Sprague Dawley rats (n=10/group). After 28 days, a significant increase in blood markers of inflammation was seen in rats exposed to all 3 extracts compared to control. A significant reduction in cholinesterase activity was observed in plasma, but not brain from rats exposed to extract A compared to control. A significant increase in hepatic ethoxyresorufin-o-deethylase activity was also observed after exposure to extracts A and B compared to control. Oxidative stress was detected in the brain and kidney tissues after exposure to extracts B and C, respectively when compared to control. Acute tubular necrosis was seen in rats exposed to all 3 extracts. Candidate causative agents include organochlorine,organophosphate/carbamate insecticides or their metabolites. Kidney damage and systemic inflammation are clear targets, but some risk may arise from brain oxidative stress. Actual human risk is unclear and must be assessed in combination with potential exposure levels.

3 - Gestational exposure to valproic acid upregulates total Stat3 protein expression while downregulating phosphorylated Stat3 in CD-1 mouse embryos with neural tube defects

Sidra Shafique[1], **Louise Winn**[1,2]
[1]**_Department of Biomedical and Molecular Sciences Queen's University, Kingston ON,_**
[2]**_School of Environmental Studies Queen's University, Kingston, ON_**

Introduction: Valproic acid (VPA), a widely prescribed antiepileptic drug and effective treatment for bipolar disorder and neuropathic pain, results in developmental defects including neural tube defects following _in utero_ exposure. Signal transducer and activator of transcription 3 (Stat3) is a transcription factor that plays an important role in cell proliferation by inhibiting apoptosis. Stat3 is activated via tyrosine phosphorylation and its subsequent dimerization results in increased nuclear Stat3 activity. Stat3, as well as its active form (pYStat3), is present in the central nervous system (CNS) during neural tube closure in murine development with implications in embryonic development. This study investigated the effects of _in utero_ VPA exposure on embryonic Stat3 mRNA and protein expression, during the critical period of neural tube closure (i.e. gestational day (GD)9) and in exencephalic embryos on GD13.

Methods: Pregnant CD-1 mice were exposed to 400 mg/kg VPA or saline on GD9 via subcutaneous injection. Embryos were harvested at 0, 1, 3, 6, or 24 hours following exposure or on GD13. Stat3 mRNA expression was measured by RT-qPCR in whole embryos at GD9 and GD10, from control embryos, VPA exposed embryos with either open or closed neural tubes (NT), and in heads of GD13 control embryos, exposed but non-affected and exencephalic fetuses. Total Stat3 and phosphorylated Stat3 levels were measured in whole cell extracts using Western blots in the same tissue and time points while in the nuclear extracts from heads of GD13 control and exposed embryos.

Results: Stat3 mRNA levels remained unchanged at all time points. Total Stat3 protein levels were significantly ($p < 0.05$) increased in GD9 embryos at 1 and 6 hours post-exposure and in GD13 heads from exposed but non-affected and exencephalic embryos. In contrast, phosphorylated Stat3 levels were significantly ($p < 0.05$) downregulated in GD9 embryos at the 3 and 6 hour time points with an overall trend of downregulation in the GD10 and GD13 groups. Total and phosphorylated Stat3 protein levels remained unchanged in nuclear extracts of GD13 heads from the exposed but non-affected and exencephalic groups.

Conclusion: The significant downregulation of phosphorylated Stat3 levels suggests that decreased Stat3 activity and resulting induction of apoptosis may lead to the failure of the neural tube to close during mouse embryo neurulation.

4 - Is there an association between proximity and density to hydraulic fracturing wells and birth outcomes?: a study in Northeastern British Columbia, Canada

Elyse Caron-Beaudoin[1], **Kristina W. Whitworth**[2], **Delphine Bosson-Rieutord**[3], **Gilles Wendling**[4], **Suyang Liu**[5], **Marc-André Verner**[1]

[1]*Department of Occupational and Environmental Health, Université de Montréal School of Public Health (ESPUM), 2375 chemin de la Cote-Sainte-Catherine, Montréal, QC H3T 1A8, Canada,* [2]*The University of Texas Health Science Center at Houston, School of Public Health, 7411 John Smith Drive, San Antonio, TX, 78229,* [3]*Department of Health Policy, Management & Evaluation (DGEPS), Université de Montréal School of Public Health (ESPUM), 7101 Avenue du Parc, H3N 1X9, Montréal, QC,* [4]*GW Solutions, Inc. 201-5180 Dublin Way, Nanaimo, BC, V9T 0H2, Canada,* [5]*Department of Global Health, School of Health Sciences, Wuhan University, 115# Donghu Road, Wuhan, 430071, China*

Background. Northeast British Columbia (Canada) is an area of intensive hydraulic fracturing for natural gas exploitation. Concerns have been raised regarding the potential health effects of contaminants emitted by gas exploitation. Some epidemiological studies have found associations between proximity/density of wells and negative birth outcomes.

Objective. Our objective was to assess associations between proximity/density of wells and birthweight, small for gestational age (SGA), preterm birth and head circumference.

Methods. We used birth records from the Fort St John hospital from January 1 2007 to December 31 2016 (n = 6333 births). We constructed three exposure metrics by calculating the inverse distance-weighted (IDW) sum of wells within three buffers around maternal postal code centroid: 2.5, 5 and 10 km. IDWs were categorized into quartiles. We then used linear or logistic regression to evaluate associations between IDW quartiles and birth outcomes. Models were adjusted for baby's sex, parity, maternal age and smoking status.

Results: We found increased adjusted odds of preterm birth associated with proximity/density of wells in the 2nd (odds ratio [95% confidence interval] = 1.60 [1.30, 2.43]) and 3rd quartiles (1.34 [0.90, 2.08]) of the 2.5-km buffer. We found significant negative associations between birthweight and proximity/density of wells in the 2nd (adjusted beta [95% confidence interval]: -40.9 g [-78.0, -3.7]) and 3rd (-42.0 g [-79.2, -4.9]) quartiles of the 5-km buffer;, and in the 3rd quartile of the 10-km buffer (-47.3 g [-84.3, -10.3]). We found no significant association with SGA or head circumference.

Conclusion. This is the first epidemiologic study in Northeastern British Columbia on the association between proximity/density of wells and birth outcomes. Our results provide some evidence of a potential association with more preterm births and reduced birthweight, but effect estimates did not match expected dose-response relationships.

5 - Consequences of in utero benzene exposure on fetal DNA repair and topoisomerase IIα in CD-1 mice

Trent Holmes[1], **Louise Winn**[1,2]

[1]**Department of Biomedical and Molecular Sciences, Queen's University,** [2]**School of Environmental Studies, Queen's University**

Evidence suggests that maternal exposure to benzene from environmental sources during fetal development may lead to hepatic and hematopoietic toxicity in offspring. Previous studies from our group have shown that _in vitro_ benzene metabolite exposures affect the critical DNA repair protein topoisomerase IIα (Topo IIα). However, a complete understanding of the impact of benzene on fetal DNA repair _in vivo_ has yet to be elucidated. This complementary _in vivo_ study investigates a potential mechanism of _in utero_ benzene toxicity in CD-1 mice which will expand upon previously determined transplacental benzene toxicity in CD-1 mice and inhibition of Topo IIα _in vitro_ following benzene metabolite exposure. Previously, _in vitro_ cultured gestational day 14 fetal liver cells showed a significant time- and dose-dependant inhibition of fetal Topo IIα after exposure to 12.5 µM of the benzene metabolite, benzoquinone. The current study will evaluate the effects of _in vivo_ maternal benzene (200 mg/kg) exposure following 2, 4, and 24 hours post dosing on Topo IIα activity. Additionally, mRNA expression of the non-homologous end joining genes _Xrcc4, Xrcc5,_ and _Xrcc6_ will be measured at 2, 4, and 24 hours post dosing. Further studies will measure levels of NHEJ proteins and develop a NHEJ activity assay to assess the functional impact of _in utero_ benzene exposure on fetal DNA repair capacity. The understanding of fetal benzene toxicity will better support informing expecting mothers on the risk of benzene exposure.

6 - Vulnerability of the maternal brain: Effects of gestational and postpartum exposure to an ecologically relevant toxicant mixture

Anne TM Konkle[1,2,3], **Sandra Konji**[1], **Claire Fong-McMaster**[4], **Amanda Nitschke**[1], **Lydia Jeong**[2]

[1]*Interdisciplinary School of Health Sciences, University of Ottawa,* [2]*School of Psychology, University of Ottawa,* [3]*University of Ottawa Brain and MInd Research Institute,* [4]*Biomedicam Science, University of Ottawa*

The developing nervous system is particularly vulnerable to the effects of environmental perturbations. The environment provided by the mother is of utmost importance; she nourishes and cleanses, and offers warmth, sensory stimulation and protection to her offspring. However, little is known about the consequences on the mother, of perturbations to the mother's own environment during pregnancy and post partum. Effects on the plasticity of her brain and consequently maternal behaviour could disrupt the environment she provides for her offspring.

Complex combinations of environmental toxicants are highly prevalent in the Canadian Arctic, placing a higher toxicant body burden on the Northern population. Twenty-seven common chemicals including polychlorinated biphenyls, organochlorine pesticides, and methylmercury (MeHg), have been used as part of an ecologically relevant toxicant mixture. The results of earlier research show the damaging effects of this mixture on fetal development, however, little research has explored its impact on maternal brain plasticity. Rat dams were treated with either vehicle, a low or high dose of either MeHg or the toxicant mixture, from conception up until postpartum day 21 at which time the brains were collected and prepared for immunohistochemistry or Nissl staining. Brain areas of interest included those directly involved in maternal behaviour - medial preoptic area and nucleus accumbens - and those indirectly involved - ventral tegmental area and hippocampus. We found no effect of treatment on body weight or surviving births, nor on the sex distribution of offspring. Brain areas studied were differentially affected by the treatments, with prominent differences found between the high and low doses specifically, in the number of glia, neurons and estrogen receptors. While functional consequences of these neuronal changes have yet to be explored, these findings suggest a vulnerability of the maternal brain to this ecologically relevant toxicant mixture.

7 - Toxicity of Aryl Phosphate Flame Retardants Mediated by Serine Hydrolase Inhibition in Rat Liver, Ovary and Adrenal Glands

Michael Wade[1], **Alice Kawata**[1], **Marybeth Creskey**[2], **Terry Cyr**[2]

[1]*Environmental Health Science & Research Bureau, Health Canada.,* [2]*Centre for Biologics Evaluation, Biologics and Genetic Therapies, Health Canada*

Exposure of rats to phosphate flame retardant (isopropylated triphenyl phosphate IPTPP) caused liver, adrenal gland and ovary enlargement. To understand the consequences and potential human relevance of these effects, we sought to identify the target molecule(s) mediating IPTPP toxicity in these organs. IPTPP was hypothesized to induce toxicity, like other organophosphates, by reacting with and inhibiting (a) serine hydrolase enzyme(s) (SHE).

Liver, adrenal glands and ovaries were collected from female rats after oral exposure to corn oil (control) or 100 mg/kg IPTPP. Tissue homogenates were reacted with a fluorophosphate bait molecule (FP) that irreversibly binds to the active site of any SHE. Initially, FP coupled with AlexaFluor 488 was used and resulting samples separated by PAGE. A difference in fluorescently labelled proteins in liver, adrenal and ovarian between control and treated samples indicated that SHEs were pre-reacted and inhibited by IPTPP. Reacted SHEs in homogenates incubated with desthiobiotin-labelled FP were concentrated with streptavidin beads. Proteins eluted after concentration were identified using LC-MS. SHE Proteins that were dramatically lower relative concentration in the IPTPP-treated animals relative to the controls were Carboxyesterase 1E (CES1E) and monoacylglycerol Lipase ABHD6 in liver and Hormone Sensitive Lipase (HSL) in both ovary and adrenal glands. IPTPP inhibited rat liver CES and the human homolog of rat CES1E (CES1) activity but the effect on the latter enzyme was slightly less (IC50 of 320 nM vs 770 nM, respectively). Studies of HSL inhibition by IPTPP and metabolites are ongoing. These studies have identified molecular targets for aryl phosphates and may provide the basis for rapid assays to compare toxicity and potential hazard across the diverse class of organophosphate flame retardants.

8 - Towards routine detection of endocrine disrupting chemicals in complex effluents in Quebec

J Robitaille[1], **M Desrosiers**[2], **I Guay**[2], **E Veilleux**[2], _Valérie Langlois_[1]

[1]*Institut national de la recherche scientifique (INRS), Centre Eau Terre Environnement, Quebec City, QC,* [2]*Ministère de l'Environnement et de la Lutte contre les changements climatiques, Quebec City, QC*

Endocrine disrupting chemicals (EDCs) are xenobiotics which have the ability to mimic or inhibit hormones. Their presence in aquatic ecosystems can affect the development and reproduction of aquatic wildlife. However, EDCs are not yet regulated in municipal and industrial wastewater in any country, including Canada. Current EDC frameworks, such as those from the United States' Environmental Protection Agency (US-EPA) and Japan's Ministry of Environment (MoE), aim to identify EDCs using a single compound approach. However, the reality of cities (as well as hospitals and industries) is to deal with complex mixtures in effluents. These effluents could yield an overall endocrine disrupting (ED) activity different from the sum of individual ED activity of each compound. This project aims to develop a two-Tiered approach for routine testing of complex wastewater mixtures which will focus on reproductive endpoints. Based on an exhaustive literature review of existing EDC frameworks, we selected three in vitro bioassays for the Tier 1: the transactivation assay of the human estrogen and androgen receptors, and the assay of steroidogenesis in H295R cells. For the Tier 2, the fish short term reproduction assay in fathead minnow was selected to validate any positive scores obtained in the Tier 1. The optimization and validation of each bioassay is currently ongoing. When fully operational, these bioassays could assist municipal, provincial, and federal governments in a first phase of testing EDCs in municipal and industrial effluents. Altogether, this long-term research program aims to better manage the quality of wastewater being released into Quebec's and Canada's ecosystems.

9 - Screening for the safety of emerging plasticizers and flame-retardants

Sarah Tardif[1], **Bernard Robaire**[2], **Géraldine Delbès**[1]

[1]**INRS, Armand Frappier Santé Biotechnologie,** [2]**McGill University, Department of Pharmacology and Therapeutic**

The use of bis(2-ethylhexyl) phthalate (DEHP) and 2,2'4,4'-tetrabromodiphenyl ether (BDE-47), belonging respectively to the phthalate and the flame-retardant families, has been regulated, in part because of their endocrine disruption activities. New chemicals that are already detected in consumer products and human matrices are replacing them. However, their potential toxicity is poorly characterized. We propose to screen the toxicity and endocrine disruptor potential of replacements chemicals and to compare their effects with those of legacy products. To this end, we use the organ culture of fetal rat testes, a tissue particularly sensitive to steroid hormone modulations. This organ-based assay is less time-consuming then _in vivo_ studies require less animals and complement _in vitro_ experiments on cell lines. This method has already been used to evaluate the effects of MEHP, the main active metabolite of DEHP. Basal and LH-stimulated testosterone secretions have been measured from culture media by ELISA. After culture, tissues have been processed for further histological analysis. Three replacements for DEHP/MEHP and two BDE-47 substitutes have been selected based on their cytotoxicity on testicular cell lines. Our results show that MEHP induces a significant decrease in basal and LH-stimulated testosterone secretions of fetal rat testes, while its replacements, 2,2,4-trimethyl 1,3-pentanediol diisobutyrate, diisononyl-phthalate and diisodecyl adipate, had no effect (n ≥ 3). In parallel, neither BDE-47 nor its substitutes, (tributoxyethyl phosphate and isopropylated triphenyl phosphate), had any impact on testosterone secretions (n ≥ 4). Preliminary histological examination after hematoxylin-eosin staining did not reveal any impact of any compound on the global architecture of the testes. More in depth analyses of cell number and proliferation rate of the main testicular cell types are ongoing using immunofluorescence. Overall, this study allows for the identification of less toxic alternatives and provides essential information regarding the need for their regulation. Funded by CIHR.

10 - Investigating Whether Epigenetic Changes in Murine Fetal Livers Occur Following Gestational Exposure to the Flame Retardant Triphenyl Phosphate

Sydney Wolpert[1], **Nicola Philbrook[2], Louise Winn[1,2]**

[1]_**School of Environmental Studies, Queen's University, ON,**_ [2]_**Department of Biomedical and Molecular Science, Queen's University, ON**_

Background: Triphenyl phosphate (TPP) is an organophosphorus flame retardant and plasticizer which is added to both industrial and consumer products. Exposure to flame retardants begins prenatally and continues throughout adulthood. It has been suggested that the gestational environment is a predictor for subsequent health and disease through epigenetic modification. Specifically, epigenetic modifications are known to alter gene expression profiles and previous work in our laboratory has shown changes in maternal gene expression, particularly levels of insulin-like growth factor, following gestational exposure to TPP. Additionally, studies have shown that other organophosphorus flame retardants are able to epigenetically alter DNA and enable histone modifications upon exposure, contributing to the development of disease. However, whether _in utero_ exposure to TPP alters epigenetics in fetal tissue remains to be determined.

Objectives: The objective of this study is to determine whether _in utero_ TPP exposure results in epigenetic modification, specifically acetylation and methylation of histone 3 (H3) and histone 4 (H4), in gestational day (GD) 19 murine fetal livers.

Methods: Pregnant C57Bl/6 mice were given intraperitoneal injection on GD 8, 10, 12, and 14 of 0, 5, 25, or 50 mg/kg TPP dissolved in corn oil. Dams were euthanized on GD 19 and fetal livers were extracted. Histones were acid extracted and 2 mg of protein were separated on a 15% polyacrylamide gel and transferred to PVDF membranes. Membranes were probed for acetylated H3, acetylated H4, and methylation at histone 3 lysine 9. Results were quantified using densitometry.

Results: Preliminary results indicate no difference in H4 hyperacetylation between fetal livers exposed gestationally to TPP compared to those exposed to corn oil. Continuing studies will determine whether other variations of histone modifications occur following TPP exposure in C57Bl/6 mice.

Conclusions: Although organophosphorus flame retardants have been shown to induce epigenetic changes, preliminary evidence suggests that TPP does not alter H4 hyperacetylation in GD19 livers of C57Bl/6 mice. This research is supported by NSERC.

11 - Investigating the effects of in utero benzene exposure on murine fetal gene expression of Xrcc5

Christian Bile[1], **Louise Winn**[1]

[1]*Department of Biomedical and Molecular Sciences, Queen's University*

Benzene is a ubiquitous pollutant found in the environment, in car emissions, cigarette smoke, and at elevated levels in the industrial manufacturing of plastics, resins and dyes. Through known exposures to benzene, it has been shown that benzene can have carcinogenic and hematotoxic effects. Currently, little is known about the effects of *in utero* benzene exposure, but it is thought that benzene and its metabolites will be more toxic to the developing fetus, since the fetus has decreased detoxification and DNA repair abilities. It has been suggested that maternal exposure to benzene *in utero* may play a role in the development of childhood leukemia. This study served to evaluate the impact of *in utero* benzene exposure on the murine gene expression of *Xrcc5* in CD-1 gestational day (GD) 14 fetal livers using qRT-PCR. The results of the study showed that there was no significant change in fetal gene expression of between the two treatment groups, control and benzene injected mice at 2 and 24 hours after final benzene dosing. There was also no significant change in fetal gene expression across the two time points. This preliminary data disagrees with data from a different adult mouse model, where the gene expression of *Xrcc5* after benzene exposure was upregulated and may indicate that the fetal response to benzene could be different than that of the adult mouse. Further analysis will include results obtained from placentas as the placenta is involved in fetal development and hematopoiesis, and can be regulated by the fetus itself. The mRNA levels of other critical genes involved in DNA repair including *Ogg1* (Bas excision repair), *Ku70/80* (Non-homologous end joining), *Rad51*, *Rad52*, *Rad54*, *Atm, Atr, Brca2* (homologous recombination) and *Brca1*, *Parp-1* and *p53* (involved in several DNA repair pathways) can also be measured using qRT-PCR. We will also evaluate sex differences to determine potential sex-dependent benzene-initiated alterations on fetal DNA repair.

12 - Using in vitro polybrominated diphenyl ether (PBDE) neurotoxicity data to establish acceptable exposure levels for developing children

Sherri Bloch[1], **Charu Chandrasekera**[2], **Nynke I. Kramer**[3], **maryse bouchard**[1], **Marc-André Verner**[1]

[1]***Université de Montréal,*** [2]***Canadian Centre for Alternatives to Animal Methods,*** [3]***Utrecht University***

Current acceptable exposure levels are mostly based on animal models, which are costly, time-consuming and may poorly predict adverse outcomes in humans. There is a need for alternative testing methods that are faster, cheaper, and provide human-relevant information. Our objective was to evaluate a method using human *in vitro* data and biological modeling to calculate an acceptable exposure level through a case study on PBDE developmental neurotoxicity. Using data from a study on human neuroprogenitor cells, we derived a point of departure using benchmark dose modeling for BDE-99 induced alteration of differentiation. We calculated a lower bound for the benchmark dose (BMDL) of 0.0832 µM for a benchmark response of 10%. We subsequently translated this BMDL expressed in terms of nominal concentration into a cellular level (2819 µg/kg cells) using the empirically-derived enrichment factor (60). We estimated the acceptable maternal daily intake (105 ng/kg/d) and plasma concentration (150 ng/g lipids) associated with a concentration of 2819 µg/kg in the child brain through reverse dosimetry using a pharmacokinetic model of gestational and lactational exposure. Finally, we compared the estimated maternal plasma BDE-99 level during pregnancy to median and maximum levels measured in epidemiological studies reporting associations with child neurodevelopment. Studies reported median levels ranging from <3.3 to 4.5 ng/g lipids, and maximum levels ranging from 169 to 298 ng/g lipids. Overall, the acceptable exposure level derived from in vitro data was higher than median levels measured in epidemiological studies, but in the range of maximum levels. Results suggest that factors related to the duration of exposure and interindividual variability may need to be taken into account.

13 - Acetaminophen (APAP) may interfere with the human fetal ovary development in an ex vivo model.

L. Lecante[1], **S. Leverrier-Penna[1], Giton F[2], V. Lavoué[3], A.D. Rolland[1], S. Mazaud-Guittot[1]**

[1]*Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) UMR-S 1085, F-35000 Rennes, France,* [2]*AP-HP, Pôle Biologie –Pathologie Henri Mondor, Créteil 94000, France,* [3]*CHU Rennes, Service Gynécologie et Obstétrique, F-35000 Rennes, France*

Taken by more than half of pregnant women, acetaminophen (APAP) is the most commonly used over-the-counter analgesic during pregnancy. Although long considered as safe, studies have showed that APAP intake may be associated with troubles in children exposed *in utero* and notably, a higher risk of male genital tract abnormalities likely due to effects on fetal testis function. Although rodent studies suggest that fetal exposure may have long-term effects on the female reproductive function, little work has been devoted to its potential effects on human ovary development.

Provided that disruption of fetal ovarian development may impact woman reproductive health, we investigated whether exposure to APAP interferes with female gonad development. Human fetal ovaries (7-12 developmental weeks (DW)) were cultured for 7 days with APAP (10^{-8} to 10^{-3}M).

APAP reduced total cell number in a non-linear manner in 10-12 DW samples, but not in 7-9 DW ones. While it did differentially alter cell viability regarding fetal age and concentration considered, APAP decreased cell proliferation percentage whatever concentration and, at 10^{-7} and 10^{-6}M, in the 8-9 DW and 10-12 DW samples, respectively. Although APAP did not impact specifically the percentage of M2A-positive germ cells, except at very high concentration, it decreased their total number, suggesting a non-specific effect on germ cells. Eight to 12 DW human fetal ovaries displayed a steroidogenic activity that was disrupted by increasing APAP concentrations, while inhibin B levels were not altered.

Our data suggest that APAP may impact human fetal ovarian development during the first trimester not only by interfering with developmental processes but also by impacting gonad endocrine function. However, crucial events for establishing the ovarian pool occur during the following months of pregnancy making it necessary to carry out further. Characterizing paracetamol effects would allow physicians to recommend better pregnant women.

14 - The role of endothelial-targeted PPARγ in mammary tumourigenesis and mammary tumour angiogenesis.

Shi JY[1], **Skelhorne-Gross GE**[2], **Rubino RE**[3], **Peterson NT**[3], **Wyndham C**[2], **Schneider M**[2], **SenGupta SK**[2], **Lebrun DP**[2,3], **Ren YMK**[2], **Reid AL**[1], **Nicol CJB**[1,2,3]

[1]*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada K7L 3N6.,* [2]*Department of Pathology and Molecular Medicine, Richardson Laboratories, 88 Stuart St., Queen's University, Kingston, Ontario, Canada K7L 3N6.,* [3]*Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University, 10 Stuart St., Rm 317, Kingston, Ontario, Canada K7L 3N6*

Peroxisome proliferator-activated receptor (PPAR) γ plays a role in tumourigenesis. PPARγ is expressed in many mammary associated cell types. Previously, we showed PPARγ suppresses 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast tumour progression in a cell type specific manner. Based on reports suggesting *PPARγ* ligands block angiogenesis *in vitro*, we hypothesized PPARγ expression and signaling within endothelial cells (ECs) is protective against mammary tumourigenesis via inhibiting mammary tumour angiogenesis *in vivo*. Female EC-targeted *PPARγ* knockout (*PPARγ*[E-KO]) and congenic wildtype (WT) mice were treated with DMBA to induce mammary tumourigenesis, and then maintained on a normal chow diet or one supplemented with the PPARγ activating ligand rosiglitazone (ROSI) and monitored weekly for up to 25 weeks. Here we show co-treatment with ROSI significantly improved tumourigenic outcomes only among controls, but not *PPARγ*[E-KO] mice, suggesting an EC-specific *PPARγ* dependent effect. Western analysis revealed that signaling changes differ between *PPARγ*[E-KO] and WT mice irrespective of treatment. Interestingly, *PPARγ*[E-KOs] had a significantly higher DMBA-induced thymic tumour incidence compared to WT controls, which was not altered by ROSI co-treatment. Investigations are currently underway for immunological differences in mammary and thymic tumours from *PPARγ*[E-KO] and WT mice. Orthotopic engraftment of lung-metastatic mammary tumour cells also resulted in more incidences of lung metastases in *PPARγ*[E-KO] than WT mice. Angiogenic and immunological changes within the orthotopic engraftments are being examined. Our *in vitro* studies also show PPARγ expression or absence did not significantly alter the inherent ability of ECs to sprout, migrate or invade. In contrast, early *in vitro* angiogenic events were significantly decreased by ROSI in WT but not *PPARγ*[E-KO] ECs. Together, our data provide the first direct *in vivo* evidence that EC-targeted loss of PPARγ increases DMBA-mediated breast and thymic tumourigenesis, and that ROSI activates PPARγ-dependent anti-cancer effects in ECs that may be critical during early angiogenic events. Our data also support a novel breast cancer therapeutic role for PPARγ activating drugs and suggest stromal PPARγ expression may be a novel predictive biomarker for improved clinical outcomes among a subset of breast cancer patients.

15 - Human Red Blood Cell Uptake and Sequestration of Arsenite and Selenite: Evidence of Seleno-bis(S-glutathionyl) Arsinium Ion Formation in Human Cells

Gurnit Kaur[1,2], **Warda Javed**[2,3], **Olena Ponomarenko**[4], **Diane P. Swanlund**[2,3], **Michael Hu**[3], **Kelly L Summers**[4,5], **Angela Casini**[6,7], **Margot Wenzel**[6], **Joseph R. Casey**[2,3,8], **Emmanuelle Cordat**[2,3], **Ingrid J. Pickering**[4,5], **Graham N. George**[4,5], _Elaine M Leslie_[1,2,3]

[1]*Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Canada,* [2]*Membrane Protein Disease Research Group, University of Alberta, Canada,* [3]*Department of Physiology, University of Alberta, Canada,* [4]*Department of Geological Sciences, University of Saskatchewan, Canada,* [5]*Department of Chemistry, University of Saskatchewan, Canada,* [6]*School of Chemistry, Cardiff University, UK,* [7]*Department of Chemistry, Technical University of Munich, Germany,* [8]*Department of Biochemistry, University of Alberta, Canada*

Over 200 million people worldwide are exposed to the proven human carcinogen, arsenic, in contaminated drinking water. In laboratory animals, arsenic and the essential trace element, selenium, can undergo mutual detoxification through the formation of the seleno-bis(*S*-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$, which undergoes biliary and fecal elimination. $[(GS)_2AsSe]^-$, formed in animal red blood cells (RBCs), sequesters arsenic and selenium, and likely slows the distribution of both metalloids to the liver, and other organs susceptible to toxic effects. In human RBCs, the influence of arsenic on selenium accumulation, and vice versa, is largely unknown. The aims of this study were to characterize arsenite (As^{III}) and selenite (Se^{IV}) uptake by human RBCs, to determine if Se^{IV} and As^{III} increase the respective accumulation of the other in human RBCs, and ultimately to determine if this occurs through the formation and sequestration of $[(GS)_2AsSe]^-$. $^{75}Se^{IV}$ accumulation was inhibited by 4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid (H_2DIDS) (IC_{50} 1 ± 0.2 μM), suggesting uptake is mediated by the erythrocyte anion-exchanger 1 (AE1 or Band 3, gene *SLC4A1*). HEK293 cells overexpressing AE1 showed concentration-dependent $^{75}Se^{IV}$ uptake. $^{73}As^{III}$ uptake by human RBCs was temperature-dependent and partly reduced by aquaglyceroporin 3 inhibitors. As^{III} increased $^{75}Se^{IV}$ accumulation (in the presence of albumin) and Se^{IV} increased $^{73}As^{III}$ accumulation in human RBCs. Near-edge X-ray absorption spectroscopy revealed the formation of $[(GS)_2AsSe]^-$ in human RBCs exposed to both As^{III} and Se^{IV}. The sequestration of $[(GS)_2AsSe]^-$ in human RBCs potentially slows arsenic distribution to susceptible tissues and could reduce arsenic-induced disease.

16 - Effects of environmentally-relevant mixtures of organophosphate ester (OPE) flame retardants on KGN cells, a human granulosa cell line

Xiaotong Wang[1], **Barbara F. Hales**[1], **Bernard Robaire**[1,2]

[1]*Departments of Pharmacology and Therapeutics, McGill University,* [2]*Departments of Obstetrics and Gynecology, McGill University*

OPE flame retardants are found ubiquitously in the environment. Previous studies suggest that exposure to OPEs may be detrimental to female fertility in humans. However, no experimental information is available on the effects of OPE mixtures on ovarian granulosa cells, which play key roles in female reproduction. Here, we tested the hypothesis that environmentally relevant OPE mixtures will adversely affect the function of granulosa cells. KGN immortalized human granulosa cells were exposed for 48h to one of three OPE mixtures. The first (total mixture) was composed of 13 OPEs detected in Canadian house dust; the second triaryl-OPE mixture, was a subset of 7 OPEs with 3 phenyl moieties in their structure; the third nontriaryl-OPE mixture contained the remaining chemicals in the total mixture. Cells were exposed to vehicle or 1/1,000,000X – 1/3,000X dilutions of these mixtures, where 1X represents the OPE concentrations in 6.32g of dust. Effects on cell survival, lysosomes, production of reactive oxygen species (ROS), and lipid droplets were determined using fluorescent dyes and high content imaging. At dilutions of 1/10,000X, the total, triaryl, and nontriaryl mixtures decreased cell survival by 80.2%, 28.6%, 52.5%, respectively. At non-toxic dilutions, only the triaryl-OPE mixture decreased the number of lysosomes per cell; the total mixture and the non-triaryl mixture increased ROS production in cells. The total, triaryl, and nontriaryl mixtures significantly increased the number of lipid droplets at exposures ≥1/300,000X, 1/1,000,000X, 1/100,000X, respectively; the total mixture induced this increase to a greater extent. Interestingly, the triaryl and nontriaryl mixtures slightly deceased the average size of individual lipid droplets in cells, whereas the total mixture increased the size at all dilutions tested. Together, these data show that the total, triaryl, and nontriaryl OPE mixtures affect specific endpoints differentially. We propose that exposure to "house dust" OPE mixtures may adversely affect female reproductive health by altering the function of granulosa cell. Supported by CIHR, McGill University, Macau Government. We thank Dr. Mike Wade (Health Canada) for preparing and providing these mixtures.

17 - An environmentally relevant mixture of organophosphate ester flame retardants negatively impacts endochondral ossification

Han (Aileen) Yan[1], **Barbara Hales**[1]

[1]*Department of Pharmacology and Therapeutics, McGill University*

Flame retardants are applied to many consumer goods to slow their burning. With the global phase out of polybrominated diphenyl ether (PBDE) flame retardants, organophosphate esters (OPEs) have taken over the market. OPE levels in the environment have quickly surpassed those of PBDEs, but little is known about the safety of these new generation flame retardants. We previously showed that several individual OPEs, such as triphenyl phosphate (TPHP) and *tert*-butylphenyl diphenyl phosphate (BPDP), were detrimental to bone formation in the *ex vivo* mouse limb bud culture model. However, real life exposure often involves mixtures. As such, in the current study, we investigated whether exposure to an environmentally relevant mixture of OPEs, whose composition is based on dust, a major route of FR exposure, could affect bone formation. We tested this using the 6-day limb bud culture system and a strain of transgenic mice expressing fluorescently tagged collagen markers for the different stages of endochondral ossification: COL2A1-eCFP (chondrogenesis), COL10A1-mCherry (chondrocyte hypertrophy), and COL1A1-eYFP (osteogenesis). Limbs from gestation day 13 embryos were cultured in the presence of vehicle (DMSO), 1/1,000,000, 1/600,000, or 1/300,000 dilutions of the OPE mixture. Limb morphology scoring indicated that exposure to even the 1/1,000,000 dilution significantly decreased the extent of cartilage template development, compared to controls. The fluorescent markers also revealed that exposure to as low as the 1/600,000 dilution inhibited the progression of bone formation. In agreement with this observation, treatment with the OPE mixture dilution-dependently reduced the mRNA expression of two master regulators that drive the later stages of endochondral ossification, *Runx2*, and *Sp7*. This is the first evidence that an environmentally relevant mixture of OPEs may be detrimental to endochondral ossification.

18 - Tungsten Alters DNA Damage Repair Mechanisms

Rowa Bakadlag[1,2]**, Hsiang Chou**[1,2]**, Steven Findlay**[1,2]**, John Heath**[1,2]**, Koren Mann**[1,2,3]

[1]**Lady Davis Institute for Medical Research,** [2]**Division of Experimental Medicine,** [3]**Gerald Bronfman Department of Oncology**

Tungsten was linked to several pediatric pre B lymphocytic leukemia clusters but was not designated as the causative agent. Tungsten increases DNA damage and gH2AX levels in developing B cells _in vitro_ and _in vivo_. B cell development depends on DNA damage and its subsequent repair by non-homologous end joining (NHEJ) to facilitate immunoglobulin recombination. Thus, we hypothesize that tungsten impedes DNA damage repair through the NHEJ pathway. RNA sequencing of murine pre B cells exposed to tungsten _in vivo_ showed decreased expression of several members of the NHEJ pathway. In addition, tungsten enhances neocarzinostatin-induced DNA-damage as assessed by increased gH2AX through flow cytometry, and may reduce repair. We further showed that tungsten abrogates NHEJ repair and demonstrated that tungsten decreases NHEJ-dependent class switch recombination to IgA. Together, our data suggest that tungsten inhibits NHEJ resulting in increased DNA damage in B cells.

19 - Increase of the adult mammary gland weight after in utero and lactational exposure to Di(2-ethylhexyl) phthalate (DEHP): a complex story

Bélinda Crobeddu[1], **Elise Kolasa**[1], **Bernard Robaire**[2], **Isabelle Plante**[1]

[1]*INRS – Centre Armand-Frappier Santé Biotechnologie, Laval, H7V 1B7, Canada,*
[2]*Department of Pharmacology and Therapeutics, McGill University, Montréal, H3G 1Y6, Canada*

DEHP is a plasticizer to which human are chronically exposed. It has been detected in different fluids such as umbilical cord blood and breast milk. DEHP is defined as an endocrine disruptor (ED), that could dysregulate mammary gland development. Furthermore, it has been shown that an exposure to ED during sensitive windows, such as *in utero* and early life, could lead to pathologies later-on in adulthood. Perinatal exposure to DEHP has also been associated with metabolic syndrome that involves adipogenesis-related pathways.

To investigate the consequences of DEHP exposure, female rats were exposed by gavage during pregnancy and lactation, and the mammary glands of their offspring were collected at weaning (PND21), during puberty (PND42) and at adulthood (PND60). An increase in the ratio of mammary gland/body weight was found at adulthood, without any modification of the epithelium surface, suggesting an increase of the fat pad surrounding the epithelium. This hypothesis was confirmed by a microarray analysis and *in silico* analysis that showed an upregulation of transcripts associated with adipogenesis, potentially through the peroxisome proliferator-activated receptor (PPAR) pathway. Surprisingly, the increased expression of PPARg was confirmed at the protein levels, but no modulations of the PPAR isoforms transcripts were observed at adulthood. To determine if the adipogenesis pathway was activated, we then investigated the downstream genes of PPAR. Interestedly, we observed a significant downregulation of *Scd1*, *STAT5a* and *Orl1*. Furthermore, mRNA levels of the retinoid X receptor (*RXR*), the co-activator of the PPAR pathway, was also downregulated. Those preliminary results are in contradiction with the microarray analysis, suggesting a more complex story than previously thought to explain the increased mammary gland weight. Further analysis of the mammary gland wholemount are ongoing to quantify more precisely the mammary gland epithelium density and ramification.

Together, these results suggest that an exposure to DEHP during the *in utero*-early life period affect the mammary gland weight and the expression of genes involved in adipogenesis at adulthood. The mechanisms linked to these dysregulations and the consequences remained to be determined.

20 - Potential role for endocrine disrupting chemicals in the etiology of postpartum depression

Alexandra Ogilvie[1,2,3], **Anne TM Konkle**[1,2,3], **Karen P Phillips**[1]

[1]_**Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa,**_ [2]_**School of Psychology, Faculty of Social Sciences, University of Ottawa,**_ [3]_**University of Ottawa Brain and Mind Research Institute**_

Postpartum depression (PPD) is a severe mental health condition that affects the health of the mother, her relationship with her child as well as the family unit as a whole. This affective disorder is likely caused by interactions between biological, environmental, and psychological factors. The aim of this conceptual analysis is to investigate and describe the state of the science that supports a role for endocrine disrupting chemicals (EDCs) in modulating hormone-dependent mechanisms underlying PPD. During pregnancy, at parturition and during the postpartum period women experience dramatic fluctuations in reproductive hormone levels and a decline in hypothalamic-pituitary-adrenal (HPA) axis functioning. Changes in gonadal and adrenal hormone levels, together with psychosocial risk factors, such as stressful life events and a history of mental health issues, increase the odds for PPD. Moreover, it is becoming increasingly apparent that women are differentially vulnerable to the effects of EDCs during periods of hormonal fluctuations. It is then plausible that EDCs may modulate gonadal and adrenal hormone-mediated mechanisms underlying PPD. Although there is evidence to support a role for EDCs in the etiology of PPD, the mechanisms underlying EDC action in the biological systems implicated in PPD requires further investigation. Such research would advance the current understanding of endocrine action in the reproductive and stress axes, as well as provide a basis for the effect of peripartum EDC exposure on maternal mental and physical health.

21 - Early immune changes induced by clozapine: potential insights into the mechanism of idiosyncratic drug-induced agranulocytosis

Samantha Sernoskie[1], **Alison Jee**[1], **Jack Uetrecht**[1]

[1]**University of Toronto**

Background. Clozapine (CLZ) is highly efficacious in the treatment of schizophrenia, but its use is limited by the risk (0.5-1%) of idiosyncratic drug-induced agranulocytosis (IDIAG). The mechanism of IDIAG is not well understood; however, like many idiosyncratic drug reactions, it is believed to involve an adaptive immune response against the reactive metabolite of CLZ. Most patients starting CLZ experience an innate immune response, evidenced by paradoxical neutrophilia and increased proinflammatory mediators including IL-6, C-reactive protein and granulocyte-colony stimulating factor, and this response resolves with continued treatment. Hypothesis. This innate immune response is necessary, but not sufficient, to produce the adaptive immune response that is responsible for IDIAG. This innate immune response may be initiated by inflammasome activation. Methods and Results. Female Sprague Dawley rats (200-250g) were administered a single i.p. dose of CLZ (30 mg/kg), and immune changes were evaluated 90 minutes and 3 hours post-injection. Differential blood counts at 3 hours revealed an increased neutrophil count, but an overall drop in leukocyte count, driven by a decrease in lymphocytes. This was preceded by increased protein levels of Il-6, IL-1β (a major product of inflammasome activation), and CXCL-1 (a neutrophil chemoattractant) at 90 minutes, measured in spleen and bone marrow homogenates by ELISAs. Increased IL-1β and CXCL-1 plasma levels were also detected by 3 hours. Using an enzyme activity assay, caspase-1 activity was found to be elevated in the spleen post-CLZ, another indication of inflammasome activation. By immunohistochemical staining, myeloperoxidase, an enzyme found in neutrophils, monocytes, and macrophages, was increased in the spleen, which suggests that the spleen is critical in induction of this early immune response. Conclusions. Through further characterization of the early immune changes that occur, we may elucidate the mechanism responsible for the onset of IDIAG, and potential options to reduce or prevent its occurrence. Preliminary results suggest that inflammasome activation may contribute to the first wave of neutrophilia observed in our rat model. Further work is required to investigate the resolution of the innate immune response and its contribution to the start of IDIAG.

22 - In utero and lactational exposure to an environmentally relevant mixture of brominated flame retardants induces precocious development of the mammary glands

Rita-Josiane Gouesse[1], **Elham Dianati**[1], **Mélanie Lavoie**[1], **Michael G. Wade**[2], **Barbara F. Hales**[3], **Bernard Robaire**[3,4], **Isabelle Plante**[1]

[1]*INRS, Centre Armand-Frappier Santé Biotechnologie, Laval, QC, Canada,* [2]*Health Canada, Environmental Health Science and Research Bureau, Ottawa, ON, Canada,*

[3]*McGill University, Faculty of Medicine, Department of Pharmacology & Therapeutics, Montréal, QC, Canada,* [4]*McGill University, Faculty of Medicine, Department of Obstetrics & Gynecology, Montréal, QC, Canada*

Mammary gland development begins during embryonic life and continues postnatally under hormonal regulation. Due to the critical role of hormones in regulating early development, this process is especially vulnerable to the effects of endocrine disruptors (EDs). Brominated flame retardants (BFRs) are chemicals added to household objects to reduce their flammability, they also act as EDs. Because they are non-covalently bound to theses objects, BFRs leach into house dust resulting in ubiquitous human exposure including of pregnant women and very young children. *We hypothesize that early exposure to BFRs can affect mammary gland development due to ED activities.* We have previously shown that gestational and lactational exposure to an environmentally relevant dose of BFRs found in house dust alters cellular interactions, thyroid hormone homeostasis and the proliferation-apoptosis balance in mammary glands of female pups at puberty. *Here we assessed whether BFR-induced disruption of mammary gland development is apparent earlier in life.* Female rats were exposed prior to mating and throughout pregnancy and lactation to mixtures based on the relative levels of BFRs found in house dust. The diets were designed to deliver nominal doses of 0 (control), 0.06 (corresponding to maximum childhood exposure), 20 or 60 mg/kg/day. Female offspring were euthanized at weaning (postnatal day 21) and mammary glands were collected. Morphological analyses showed that the lowest dose of BFRs significantly increased total mammary epithelial surface ($p < 0.05$). This was associated with a trend towards an increase in both duct area ($p = 0.09$) and nuclear localization of the proliferation marker KI67 ($p = 0.09$). Finally, the low-dose exposure significantly upregulated the protein levels of p-Cx43, a gap junctional marker and cleaved caspase-9, an apoptosis marker ($p < 0,05$). These findings are characteristic of the pubertal development of the mammary glands; however, classical markers of puberty (vaginal opening and cyclicity) were not affected. Thus, our results suggest that exposure to the low dose of an environmentally relevant mixture of BFRs induces precocious development of the mammary glands independent of central reproductive maturation. Supported by FRQS, QBCF, NSERC (IP), CIHR (ML, BFH, BR), RQR-CIRD (RJG) and FAF (ED, RJG).

23 - Predicting the placental transfer of chemicals using quantitative structure-activity relationship (QSAR) modeling

Laura Lévêque[1,2], **Nadia Tahiri**[1,2], **Marc-André Verner**[1,2]

[1]**Department of Occupational and Environmental Health, School of Public Health, Université de Montréal, Canada,** [2]**Center for Public Health Research, Montréal, Canada**

Risk assessment of prenatal exposure to chemicals is paramount considering the potential adverse effects on the developing organism. To date, few tools are available to predict the placental transfer of chemicals across the placenta, especially for environmental chemicals. The aim of this study was to develop a QSAR model predicting cord-maternal plasma concentration ratios for environmental chemicals. We compiled cord-maternal plasma concentration ratios from published articles on multiple environmental chemicals including polychlorinated biphenyls, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and pesticides. We also included cord-maternal plasma concentration ratios for certain drugs to expand the domain of applicability. Only ratios from studies where cord and maternal blood samples were collected within 24 hours were kept in the dataset (n=88). We calculated 1614 descriptors for each chemical using the Mordred software. A suite of predictive models were developed through 10-fold cross-validation using 80% of the chemicals. Models were then tested against the remaining 20% of chemicals (external dataset). All statistical analyses were performed using the Sklearn package in Python. Cord-maternal plasma ratios ranged from 0.05 to 3.05 (median: 0.52), indicating that some chemicals preferentially partition into maternal or fetal plasma during pregnancy. The model developed using random forest displayed the greatest precision, with a coefficient of determination (R^2) of 0.98, a cross-validation R^2_{cv} of 0.89, and an external validation R^2_{ext} of 0.87. This level of precision was among the highest achieved for the placental transfer of chemicals and drugs. Using this QSAR model will help quantify fetal exposure to chemicals based on measured maternal plasma levels, or through the parameterization of physiologically-based pharmacokinetic models of pregnancy.

24 - Air Pollution and the Stress Axis: Regulation of Tryptophan Metabolism, Serotonin Receptor and Neurotrophic Factor Expression

Mercedes Rose[1,2], **Alain Filiatreault**[1], **Andrew Williams**[1], **Josée Guénette**[1], **Errol Thomson**[1]

[1]**Health Canada**, [2]**University of Ottawa, Faculty of Medicine**

While an increasing literature supports a relationship between air pollution and neurological diseases, the mechanistic underpinnings remain unclear. Stress hormones are strongly associated with neurological disease outcomes, causing alterations in cognitive and behavioural function as well as biochemical pathways contributing to disease progression. Previously, air pollution exposure has been shown to elevate levels of stress hormones through hypothalamic-pituitary-adrenal (HPA) axis activation. This suggests a potential mechanism through which air pollution acts to induce neurotoxic changes; however, little research has been conducted to investigate this. This study's objective was to determine whether ozone could cause alterations in hippocampal (a stress-sensitive region of the brain) and systemic pathways relevant to neurological disease. Specifically tryptophan metabolism, serotonin signaling, and expression of neurotrophic factors, all of which are often associated with neurological disease outcomes such as depression, were assessed. We also sought to establish the role of the HPA axis in mediating these effects. Male Fischer-344 rats (n=5/group), treated with or without metyrapone, an inhibitor of glucocorticoid synthesis, were exposed by nose-only inhalation for 4 hours to either air or ozone (0.8 ppm). Following ozone exposure, an increase in kynurenine, a tryptophan metabolite, in plasma was detected (2-fold, $p<0.001$). Differential gene expression of serotonin receptors and a decrease in neurotrophic factors within the hippocampus were found. Effects were mediated in both stress-dependent and independent manners. Our study reveals that ozone exposure impacts mechanisms relevant to neurological disease and suggests that the HPA axis may be intricately involved in mediating these effects. These findings help further our understanding of the many complex ways in which air pollution impacts human health.

25 - DNA methylation dynamic in rat male germ cell during gametogenesis and spermatogenesis

Rhizlane El omri[1], **Mariana Gabriela Ghinet**[2]**, Claude Robert**[3]**, Guylain Boissonneault** [2]**,
Géraldine Delbes**[1]

[1]_INRS- Armand Frappier Santé Biotechnologie, Laval, Canada,_ [2]_Department of
Biochemistry, Université de Sherbrooke, Sherbrooke, Canada,_ [3]_Department of Animal
Sciences, Université Laval, Québec, Canada_

In mammals, DNA methylation profiles in male germ cell display dynamic changes from fetal germ cells to mature spermatozoa. This establishes the sperm epigenome that will in part guide embryo development. Importantly, DNA methylation has been shown to be under the influence of environmental toxicants (ET), potentially leading to abnormal cell differentiation, function and infertility. However, we still lack the comprehensive understanding of the establishment of DNA methylation during perinatal life and spermiogenesis, especially in rats, the preferred animal model used in toxicology. One major limitation has been the purification of male germ cells at different stages during perinatal life and spermiogenesis. In this study, we aim to provide a precise developmental map of the male germline methylome. To obtain purified germ cells at different stages of development, we took advantage of a unique transgenic rat model in which germ cells exclusively express green fluorescent protein. This, combined to ploidy and DNA compaction, allowed us to purify 8 germ cells populations by fluorescent-activated cell sorting: a: gonocytes from gestational day 16 (GD16); b: gonocytes from GD20; c: spermatogonia from postnatal day 5; d: spermatids at stages 1-9; e: spermatids 10-12; f: spermatids 13-14; g; spermatids 15-17; h: mature sperm. Following DNA extraction, methylation will be assessed with the commercialy available rat Methyl-Seq platform which targets promoters, CpG islands, island shores, and GC-rich regions from all RefSeq genes. Data obtained from sequencing will be analyzed to identify differentially methylated regions between the various stages. Thereafter, this reference methylome will be used to assess the sensitivity of male rat germ cell to ET. The proposed study will provide the first detailed description of the DNA methylome landscape in the male germline throughout development, and identify epigenetic signatures of exposure to environmental chemicals. Funded by FRQNT 2019-PR-253559

26 - Optimization of a 3D co-culture model and bilayered co-cultured model as biologically representative alternatives for mammary gland toxicology.

Melany Juárez[1], **Anne Weber-Ouellette[1], Alec Mcdermott[1], Isabelle Plante[1]**

[1]*INRS-Institut Armand-Frappier*

The functional unit of the mammary gland is the bilayered acinus, composed of an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells. Evidence has shown that the *in vivo* tridimensional (3D) environment and the bidirectional crosstalk between cells are crucial for proper function, development and differentiation of the mammary gland. Our project aims to develop a representative model *in vitro* of the interactions between luminal and myoepithelial cells of the mammary gland. This is achieved by establishing a 3D bilayered co-culture model and a 2D layered co-culture model using both types of cells. Both the composition of the extracellular matrices and of the types cell lines were optimized. Our results show that co-cultured luminal MCF-12A and myoepithelial-like Myo1089 human cells, in Matrigel, form bilayered acini. Similarly, when luminal MCF-12A cells and myoepithelial-like Hs578Bst cells are co-cultured in Matrigel, few bilayered acini are formed. However, monolayered acini made of only luminal cells were abundant. Notably, these 3D models closely mimic the structure of the bilayered acini observed *in vivo*, thus suggesting that they could represent an acceptable *in vitro* surrogate to the *in vivo* animal models.

In parallel, MCF-12A and Myo1089 cells were co-culture, each on a different side of inserts with porous membranes to create a layered cultured system. Our results have demonstrated that cells seeded on each side of the membrane can communicate via gap junctions, as demonstrated by dye transfer assays using calcein and DiL. Immunofluorescence analysis suggested that junctions are formed between luminal and myoepithelial cells likely through cell projections within the pores of the membrane. Future experiments will aim to evaluate the impact of bidirectional crosstalk on cell signaling in each cell type. We expect our innovative *in vitro* models will provide a more biologically relevant option for toxicology studies through mimicking the structure, the communication and the composition of the mammary gland. Accordingly, filling the gap between results obtained from laboratory models and the expected results from animal testing. Project funded by FRQS, QBCF, NSERC (to IP), MITACS (to MJ) and FAF (to MJ, AWO and AM).

27 - Binding Partners of Glutathione Transferase GSTT1

Amy Hoff[1], **P. David Josephy**[1]

[1]**Molecular and Cellular Biology, University of Guelph**

Background

Glutathione Transferase Theta 1 (GSTT1) is a member of the GST superfamily. GST enzymes are found in almost all organisms; they protect cells from damage due to electrophilic (mutagenic/carcinogenic) compounds, catalyzing their conjugation with the non-protein thiol, glutathione. Several properties of human GSTs are unusual. The enzyme has a relatively low catalytic activity. A homozygous null GSTT1 genotype is present in about one-third of the population. Although GSTs are regarded as enzymes of xenobiotic detoxication, GSTT1 expression is higher in prostate than in liver or kidney.

Objectives

We hypothesize that GSTT1 has a "moonlighting" function independent of its catalytic activity. Recent studies have shown that several other GSTs, notably GST Pi, have binding interactions with signalling proteins (e.g. TRAF4, JNK, ASK1) that have been linked to anti-cancer drug resistance in tumors. We are identifying possible binding partners (protein-protein interactions) of GSTT1, which could provide clues to a role in signalling or other processes.

Methods and Results

Purified recombinant his-tagged GSTT1 was used as bait in "pull-down" experiments with cell lysates prepared from cultured human prostate cells and human erythrocytes. Following trypsin digestion of elution proteins, proteomic mass spectrometry was performed to identify possible binding partners. Preliminary results have identified several potential binding partners, including HINT1. The HINT1 gene has been identified as a tumour suppressor; mutations in HINT1 are associated with hereditary neuropathies, such as Charcot-Marie-Tooth disease.

Conclusions

Online databases suggest an interaction between HINT1 and GSTT1. Future research will involve western blotting, co-immunoprecipitation, and other approaches to test the possible binding interactions between HINT1 and GSTT1 proteins. We hope that these studies will identify as-yet-unknown, biologically significant roles of GSTT1.

28 - Cyanonitroanilines (Aminonitrobenzonitroles): Genotoxicity Studies; Characterization of a Novel Cyclic Deoxyguanosine Adduct

P. David Josephy[1]**, Richard Manderville**[1]**, Trevor Manning**[1]
[1]***Department of Molecular and Cellular Biology, University of Guelph***

Background

Azo (Ar-N=N-Ar') compounds are the most important class of synthetic dyes. Although the health hazards of exposure to azo dye intermediates have been known since the 19th Century, dye pollution continues to pose a threat to the environment. Azoreductase enzymes, found in many gut bacteria, catalyze the reductive cleavage of the azo bond, releasing aromatic amines. The resulting metabolites are of toxicological concern, as many aromatic amines and nitroaromatic compounds are proven mutagens and carcinogens 4-Nitroaniline (4NA) was identified as an Ames test mutagen – although a weak one - more than 30 years ago. We reported that 2-cyano-4-nitroaniline and 2,5-dicyano-4-nitroaniline are extremely strong mutagens in the Ames test, comparable in potency to nitropyrenes (*Environ. Mol. Mutagen.*, 2016 and 2018).

Results

The potent Ames test mutagenicity of cyanonitroanilines (CNAs) requires the presence of all three substituents: amino, nitro, and cyano; compounds bearing any two are no more than weakly mutagenic. We will present further studies of the mechanism by which Ames test bacteria activate CNAs. We have also synthesized C8-guanine adducts of CN-substituted anilines and we provide definitive proof that such adducts can cyclize. We suggested (2018) that the C atom of the cyano group of a putative CNA-dG adduct could react with the N7 atom of the base. The resulting cyclic adduct might be unusually repair-resistant/ pro-mutagenic. To test this possibility, we synthesized the simplest (*ortho*-cyanoaniline) C8-deoxyguanosine adduct that could form such a cyclic structure, as well as the *para* isomer, which can not. Both adducts were isolated and characterized by NMR and mass spectrometry. The data confirm cyclization of the *ortho* isomer. The adduct of the *ortho* isomer is intensely fluorescent, but that of the *para* adduct is not; and the characteristic CN band is present in the *para* (acyclic) adduct but absent in the *ortho* (cyclic) adduct.

Conclusions

· Both NAT/OAT and NR enzymes activate CNAs in the Ames test.

· CNAs can form polycyclic adducts with DNA bases; such adducts might be unusually repair-resistant or pro-mutagenic.

29 - Neutrophilia in immune checkpoint blockade mouse model of idiosyncratic carbamazepine-induced liver injury

Alison Jee[1]**, Samantha Sernoskie[2], Jack Uetrecht[1,2]**

[1]Department of Pharmacology & Toxicology, University of Toronto,[2]Leslie Dan Faculty of Pharmacy, University of Toronto

Idiosyncratic drug reactions (IDRs) are a class of adverse drug reactions that occur rarely and unpredictably and can be life-threatening. There is consensus that most IDRs are mediated by an adaptive immune response. Our hypothesis is that drugs that cause IDRs, or serious, adaptive immune responses, in a small proportion of individuals cause a subclinical, innate immune response in most patients, and even in many animals. We have chosen carbamazepine to study because it is associated with a variety of IDRs involving the liver, skin, and blood.

The immune system is, of course, extremely complex, and there are many parameters that could be investigated. Carbamazepine treatment is associated with multiple blood dyscrasias including agranulocytosis. We have previously shown that carbamazepine metabolism by myeloperoxidase, found in neutrophils and monocytes, generates several reactive metabolites, and demonstrated covalent binding of these metabolites to patient neutrophils _in vitro_. Together, these lines of evidence suggest that neutrophils, a cell type involved in the innate immune response, may be an important cell type to study. Thus, we wished to determine whether we could detect an early change in neutrophil numbers or activation state following oral administration of carbamazepine. In the present study, we treated both wildtype mice and our immune checkpoint blockade mouse model with carbamazepine, dosed by oral gavage at 75 mg/kg every 12 hours for studies up to 2 days, or 0.5% carbamazepine in food (w/w) for 1-week studies. Mice were sacrificed at early time points (3 hours, 1 day, 2 days, 1 week) and blood, spleen, and bone marrow were analyzed by flow cytometry. No changes were observed at 3 hours, 1 day, or 2 days. At 1 week, only in the immune checkpoint blockade mouse model, neutrophils and monocytes were increased in blood and spleen. No change in neutrophil activation markers was observed. Further studies will be required to determine the time course of the neutrophilia and identify the reason for the discrepancy in the wildtype and immune checkpoint blockade model response.

30 - Characterizing the low dose effects of methylmercury in early developmental stage using cultured human embryonic stem cells

Bai Li[1,2], **Xiaolei Jin**[1], **Laurie HM Chan**[2]

[1]***Regulatory Toxicology Research Center, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada**,* [2]***Department of Biology, University of Ottawa, Ottawa, Ontario, Canada***

Methylmercury (MeHg) is a ubiquitous environmental contaminant. The body of evidence available to date suggests that neurodevelopment is the most sensitive health outcome and development in utero the most sensitive period of MeHg exposure. While most *in vitro* studies have focused on the effects of MeHg exposure during neural differentiation using differentiated cells, the effects of embryonic exposure to low dose MeHg at preimplantation stage remain unclear. In this study, we used undifferentiated human embryonic stem cells (hESC) as an *in vitro* model to determine the effects of MeHg exposure at preimplantation stage. The hESC were exposed to Na_2CO_3 as vehicle control and 5-200 nM MeHg in fresh Essential 8[TM] Flex Medium on matrigel at 37 $^{\circ}$C, 4% O_2 and 10% CO_2 for 24 h or 7 days. Cell morphology and colony formation were examined under microscope. Cell viability, proliferation, apoptosis, autophagy, cell cycle, and stress response were measured at the end of exposures to MeHg. Our results revealed that exposure to nano molar concentrations of MeHg reduced cell attachment and cell viability, inhibited colony formation, impacted on cell cycle, induced apoptosis and oxidative stress, decreased autophagy, and dose-differentially altered expression of cell linage makers and pluripotent genes. These results suggest that embryonic exposure to nanomolar concentrations of MeHg can affect fetal development at the preimplantation stage.

31 - Impact of copper oxide particle solubility on lung epithelial cell toxicity: response characterization using global transcriptional analysis

Andrey Boyadzhiev[1,2], **Mary-Luyza Avramescu**[1], **Dongmei Wu**[1], **Andrew Williams**[1], **Pat Rasmussen**[1,3], **Sabina Halappanavar**[1,2]

[1]*Environmental Health Science and Research Bureau, HECSB, Health Canada,*
[2]*Department of Biology, University of Ottawa,* [3]*Earth and Environmental Sciences Department, University of Ottawa*

Metal oxide nanomaterials are ubiquitously produced and incorporated in many consumer and commercial products globally. There is a high propensity for exposure to these materials in occupational settings or through consumer products. Copper oxide nanoparticles (nano CuO) are widely used in electronics and biocidal coatings. Pulmonary toxicity resulting from nano CuO exposure is surmised to be a combination of exposure to particulate and dissolved forms of nano CuO. In the current study, *in vitro* cellular response to microparticulate, nano CuO, and dissolved CuO in the form of $CuCl_2$ was characterized using transcriptomics methods. Gene expression profiles were used to identify particle and ion specific-effects and determine whether there is a nano-specific hazard associated with this metal oxide. Lung epithelial cells (FE1) were exposed to 1-25 µg/mL micro CuO and nano CuO as well as a relevant concentration of $CuCl_2$ in submerged conditions for 2, 24, and 48 hours. Treatment with nano CuO resulted in pronounced dose and time-dependent cytotoxicity which was not seen with micro CuO or $CuCl_2$ treatments. Transcriptionally similar toxicity dynamics were seen with nano CuO and $CuCl_2$ treated cells, with induction of oxidative stress, misfolded protein, and DNA damage response pathways commonly noted. Dysregulation of cell cycle, growth, and proliferation related pathways was also commonly induced by ionic and nanoparticulate exposures. However, the effects were greatly exacerbated in response to nano CuO and progressed to apical toxicity much more rapidly compared to the cells exposed to $CuCl_2$. The kinetically rapid response to nano CuO is attributed to receptor mediated internalization and phagocytic modes of uptake which rapidly localized the stressor inside the cell. From these results, it is shown that there is a unique nanoparticulate mode of action which governs the apical toxicity of nano CuO and facilitates the effects induced by the ionic fraction.

32 - Covalent binding of trimethoprim to liver and epidermal skin proteins

Yanshan Cao[1], **Ming-Cheh Liu**[2], **Jack Uetrecht**[1]

[1]**University of Toronto,** [2]**University of Toledo**

Evidence suggests that adverse reactions are caused by reactive metabolites of the drug that covalently bind to proteins. Trimethoprim (TMP) is associated with a significant incidence of skin reactions and mild liver injury. TMP can be metabolized to two potential reactive metabolites: a reactive quinone methide and α-hydroxy-TMP, which could potentially form a reactive benzylic sulfate. There is little P450 in the skin, but there is sulfotransferase. Antibodies against TMP were used to detect covalent binding to provide clues to the mechanism of TMP-induced skin rash and liver injury.

Female Brown Norway rats were treated with 450 mg/kg/day of TMP or TMP-ketone by gavage for 3 days or with 0.5-1% of TMP in food for 5 weeks. Liver S9 and epidermal skin proteins were obtained to study covalent binding of TMP. _In vitro_, TMP, α-hydroxy-TMP, TMP-ketone or TMP-sulfate was incubated with liver S9 fraction and epidermal skin proteins isolated from Sprague Dawley rats. Western blots using a TMP-antiserum were used to detect covalent binding.

In vivo, covalent binding in the liver was observed in the rats treated with TMP, but not with TMP-ketone. This suggests that most of the binding was not due to the benzylic sulfate because it is expected that the ketone would be in equilibrium with α-hydroxy-TMP. Covalent binding in the liver appears to be due to the quinone methide formed by P450. Significant covalent binding of TMP to epidermal skin proteins was not observed in the treated rats. Our collaborator also found that α-hydroxy-TMP was a weak substrate for a fetal sulfotransferase isoform, but not other isoforms.

In vitro, TMP-sulfate is reactive and covalently binds to proteins in epidermal skin and liver S9 fractions. Incubation of α-hydroxy-TMP with epidermal skin proteins led to weak covalent binding. It appears to be sufficiently reactive to bind to proteins in the skin without further metabolism, and it may be responsible for TMP-induced skin rashes. Therefore, the metabolite responsible for skin rashes may be different from that responsible for liver injury.

33 - Dimethoxymethane: A Case Study in Applying the Hazard-based Approach to Inhalation-based Exposures in a Screening Assessment within the Chemicals Management Plan

Nazem El Husseini[1], **Khaled Himmat**[1], **Adam Griffiths**[2]

[1]**Existing Substances Risk Assessment Bureau, SED, HECSB, Health Canada, Ottawa, Canada**, [2]**Climate Change and Innovation Bureau, SED, HECSB, Health Canada, Ottawa, Canada**

The Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. The third phase of the CMP addresses 1550 remaining priority chemicals. As a part of this effort, a hazard-based approach was developed in order to determine whether a substance is considered of low concern to human health based on inherent low toxicity without the need to quantitatively characterize exposures or risk. Using a step-wise approach, available animal and human toxicity data are examined to determine the potential for serious health effects up to the OECD Limit Dose of 1000 mg/kg bw/day. For oral and dermal exposures, an absence of health effects below 1000 mg/kg bw/day in repeat-dose studies lasting 90 days or longer indicate that the substance may be of low concern for human health, provided there are no concerns of carcinogenicity, mutagenicity or reproductive/developmental toxicity; decisions on whether the approach is appropriate are based on the weight of evidence for each substance. Here we demonstrate the application of this hazard-based approach in the draft screening assessment of dimethoxymethane (DMM), which has the inhalation route as the main route of exposure. As there is no limit-dose equivalent for the inhalation route, the appropriateness of this approach is applied on a case-by-case basis. DMM is a liquid at room temperature with high vapour pressure, and has been tested in laboratory animals for up to 13 weeks in repeat-dose and developmental inhalation toxicity studies. The health effects information for this substance is discussed in the context of the approach. Following the principles of the Hazard-based Approach, DMM is considered to be of low concern for human health and the risk to the general population was not characterized quantitatively in the draft assessment.

34 - Identifying transcriptional changes in early placentation-related pathways following exposure to naphthenic acids

Laiba Jamshed[1], **Lina R. Yacoub**[1], **Genevieve A. Perono**[1], **Sergio Raez-Villanueva**[1], **Shanza Jamshed**[1], **Philippe J. Thomas**[2], **Alison C. Holloway**[1]

[1]**_Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON.,_**
[2]**_Environment and Climate Change Canada, Ottawa, ON._**

Introduction: The Alberta oil sand deposits contain 143Gt of bitumen, the extraction of which generates large amounts of residual tailings water known as oil sands process-affected water (OSPW). Naphthenic acids (NA) are considered main toxicity drivers from exposure to this complex environmental mixture. Reproductive toxicity of NA has been well established in non-mammalian vertebrates, but the effects on mammalian reproduction are less known. The goal of this study was to determine the effects of NA exposure on key pathways of mammalian placental development and function.

Methods: HTR-8/SVneo cells were exposed to 1.25 and 125 mg/L of a commercial technical NA mixture for 6h and 24h; these concentrations are within the range of NA concentrations reported in OSPW. A Nanostring® array was conducted to analyze transcriptomic changes related to placentation pathways, followed by the identification of highly dysregulated targets associated with NA treatment. Of these, growth/differentiation factor 15 (_GDF15)_ was selected for further analysis as it is highly expressed in the placenta, yet its role in placental function is not well known. We assessed the ability of NA to alter GDF15 secretion via ELISA, and the mechanism(s) of NA-mediated GDF15 transcriptional regulation through analysis of mRNA expression of transcription factors known to regulate _GDF15;_ including specificity protein 1 (_SP1_), hypoxia-inducible factor-1a (_HIF1α_), tumour-suppressor protein p53 (_p53_), activating transcription factor 3 (_ATF3_), early growth response protein 1 (_EGR1_), kruppel-like factor-4 (_KLF4_), and nuclear factor-κB (_NFκB_).

Results: NA treatment (125 mg/L) significantly altered placentation-related pathways including angiogenesis, epithelial mesenchymal transition and extracellular matrix remodelling. This same concentration of NA also resulted in a significant increase in _GDF15_, and a greater than 20% increase in GDF15 secretion (p=0.06). _KLF4, EGR1,_ and _ATF3_ all significantly increased at both 6h and 24h with high dose NA. _NFκB_ increased significantly with 125 mg NA/L at only 24h, while _HIF1α, p53_ and _SP1_ showed no effect.

Conclusions: While commercial NA mixtures are not representative of those found in OSPW, these results indicate that NA exposure perturbs placentation-related pathways. The increased secretion of GDF15, a stress-induced cytokine, and the upregulation of its transcription factors by NA may be a result of peroxisome proliferator-activated receptor γ (PPARγ) or aryl hydrocarbon receptor (AHR) mediated pathways. However, further studies are required to establish the mechanism(s) by which NA affect placental trophoblast cell gene expression.

35 - Quantitative structure-activity relationship (QSAR) modeling as a tool to assess lactational exposure for data-poor chemicals

Nadia Tahiri[1,2], **Laura Lévêque**[1,2], **Marc-André Verner**[1,2]

[1]***Department of Occupational and Environmental Health, School of Public Health, Université de Montréal,*** [2]***Center for Public Health Research, Montréal***

Chemicals are regularly detected in breast milk samples from the general population, indicating that children are exposed during lactation. However, the transfer of chemicals from maternal blood to breast milk is unknown for most chemicals on the market and their metabolites. In this study, we aimed to develop a quantitative structure-activity relationship (QSAR) model to predict milk-plasma concentration ratios for chemicals based on their chemical structural properties. We compiled published milk-plasma concentration ratios for 183 chemicals, including 110 pharmaceutical drugs and 72 environmental chemicals (e.g., polychlorinated biphenyls, dioxins/furans, organochlorine pesticides organochlorines, phenols, parabens, perfluoroalkyl substances, and several relevant metabolites). We then used the Mordred software to calculate 1614 chemical descriptors for each chemical in the database. Multiple predictive models (e.g., random forest, k-nearest neighbor, lasso regression) were developed through 10-fold cross-validation using 80% of the chemicals (internal dataset), and tested against the remaining 20% (external dataset) using the Sklearn package in Python. We estimated the performance of each model based on the coefficient of determination (R^2), cross-validation coefficient of determination (R^2_{cv}) and external validation coefficient of determination (R^2_{ext}). Milk-plasma concentration ratios ranged from 0.01 to 20.47, with a median value of 1.25. The random forest model yielded the best results in terms of R^2 (0.94), R^2_{cv} (0.52) and R^2_{ext} (0.50). Several descriptors contributed to the random forest model, including acidic and basic group counts. QSAR modeling will help estimate the lactational transfer for data-poor chemicals, namely through the parameterization of generic physiologically-based pharmacokinetic models of pregnancy and lactation.

36 - Does exposure to dibenzothiophene and its alkylated analogue, 2,4,7-trimethyldibenzothiophene, affect key pathways important for placental trophoblast cell function?

Sergio Raez-Villanueva[1], **Genevieve A. Perono[1], Philippe J. Thomas[2], Alison C. Holloway[1]**
[1]_**Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON.**_,
[2]_**Environment and Climate Change Canada, Ottawa, ON.**_

Introduction: Bitumen (thick, heavy crude oil) contains polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs, and dibenzothiophenes (DBT). In mammals, exposure to PAHs can have profound effects on male and female reproduction. However, the effects of exposure to DBT and its alkylated analogues on reproductive outcomes are unknown even though these compounds are often detected in animals exposed to petroleum-derived compounds. DBT and its alkylated analogues have been reported to alter steroid production and increase markers of oxidative stress and inflammation. These pathways impact placental trophoblast cell function and thus normal placental development and function; a key contributor to reproductive success. The goal of this study was to examine the effect of DBT and its alkylated analogue, 2,4,7-trimethyldibenzothiophene (C3DBT) exposure on steroidogenic, inflammatory, and oxidative stress pathways in placental trophoblast cells.

Methods: HTR-8/SVneo cells were exposed to DBT and C3DBT for 48h at concentrations representative of tissue levels of DBT measured in wildlife carcasses collected from the oil sands region of Northern Alberta. We assessed mRNA expression of 3β-HSD1 (_HSD3B1_; catalyzes the conversion of pregnenolone to progesterone), 17β-HSD1 (_HSD17B1_; catalyzes the conversion of androstenedione to testosterone), and P450 aromatase (_CYP19A1_; key enzyme in estrogen synthesis). To assess inflammation and oxidative stress, we measured interleukin 1β, prostaglandin-endoperoxide synthase 2 (key enzyme in prostaglandin production), and heme-oxygenase 1 (_HMOX1_; induced with exposure to oxidative stress) mRNA.

Results: DBT but not C3DBT significantly increased expression of _HSD3B1_, although there did not appear to be a clear dose-response relationship. There was no effect of DBT or C3DBT on the expression of _HSD17B1_ or _CYP19A1_ at any dose tested. Similarly, there was no effect of either compound on any markers of inflammation. DBT but not C3DBT significantly decreased expression of _HMOX1_ at all doses tested.

Conclusion: Although DBT and its alkylated analogues have been shown to affect steroid production, we saw limited effect of either test compound on the mRNA expression of key placental steroidogenic enzymes. However, this study was limited to only one C3-DBT analogue. DBT exposure had a profound effect on the expression of _HMOX1_. Heme-oxygenase 1 enzyme activity can protect placental cells against cytotoxic, inflammatory, and hypoxic insults. Therefore, the loss of _HMOX1_ expression may adversely affect trophoblast survival and/or function and lead to placental dysfunction following DBT exposure.

37 - Effect of sulphur-containing heterocyclic aromatic hydrocarbon exposure on pancreatic beta cell health

Ineli Perera[1], **Philippe J. Thomas**[2], **Alison C. Holloway**[1]

[1]**Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON.,**
[2]**Environment and Climate Change Canada, Ottawa, ON.**

Introduction: Type 2 diabetes (T2D) is the most prevalent form of diabetes and is characterized by reduced beta cell mass and function. There is increasing evidence to suggest that exposure to environmental pollutants, including polycyclic aromatic compounds (PACs) may play a role in the etiology of T2D. Although there has been considerable interest in the association between oxygen-containing PACs and the development of T2D there is little known about the toxicity of sulphur-containing PACs (S-PACs), naturally occurring chemicals found in petroleum and bitumen, on beta cell physiology. Dibenzothiophene, a commonly occurring S-PAC in petroleum has been reported to increase ROS production in mammalian cells. Importantly, pancreatic beta cells are particularly sensitive to oxidative damage and increased ROS has been shown to induce beta cell senescence. The goal of this study was to determine the effects of dibenzothiophene (DBT) and benzo[b]naphtho[2,3D]-thiophene (BNT(2,3D)), two S-PACs commonly found in bitumen on pancreatic beta cell health.

Methods: INS1E cells, a rat pancreatic beta cell line, were exposed to DBT and BNT(2,3D) for 48 hours. We assessed the mRNA expression of markers associated with antioxidant activity (_Sod2, Catalase and Nrf2_) and cellular senescence (_Glb1_ and _p21_). Next, we evaluated intracellular ROS production and cellular senescence following treatment with DBT and BNT(2,3D).

Results and Discussion: BNT(2,3D) upregulated the expression of _Sod2_, _Catalase_, _Nrf2_, _Glb1_ and _p21_, while DBT upregulated the expression of _Sod2_, _Catalase_, _Nrf2_ and _Glb1_; thereby suggesting increased antioxidant expression and cellular senescence following exposure to S-PACs. Both BNT(2,3D) and DBT significantly increased intracellular ROS levels at environmentally relevant concentrations. This increase in ROS production was associated with a significant increase in cellular senescence in BNT(2,3D)-treated, but not DBT-treated, beta cells.

Conclusion: Exposure to S-PACs significantly increases endogenous ROS production in pancreatic beta cells. Interestingly despite the fact that both BNT(2,3D) and DBT increased ROS production, only BNT(2,3D) treatment resulted in cellular senescence. This work has shown that S-PACs can perturb key pathways which are important for beta cell function. Notably, BNT(2,3D) increased cellular senescence which has been associated with impaired glucose tolerance and basal insulin secretion, suggesting that BNT(2,3D) may be a novel beta cell toxicant and may influence rates of T2D in people living in areas of high oil and gas extraction activities.

38 - Approaches for Pesticide Residue Analysis in Cannabis Products for the Canadian Market – Instrumentation and Methods

Richard Bagshaw[1]**, Mandeep Singh**[1]**, Jane Zhang**[1]**, Andy Quaile**[1]**, Albert Licollari**[1]

[1]***Nucro-Technics***

Cannabis products destined for sale in the Canadian market are required by Health Canada to be free of pesticide residues that are banned for use in the industry. Health Canada curates a list of unauthorized pest control products that include Organophosphates, Chlorinated hydrocarbons, Carbamates, and Pyethroids. While residue testing for pest control products in agricultural commodities have mature approaches that are well documented – such as USP <561> and EURL DG SANTE – applying these approaches for the testing in cannabis products in compliance with Health Canada directives are not straight forward. The main problems associated with this testing are, 1) the minimum levels of quantitation for residues challenge the sensitivity and selectivity of the GC-MS and LC-MS instrumentation and associated methods, 2) the levels of quantitation for the pest control products range almost three orders of magnitude for the listed pest control products, and 3) multiple methods and both GC-MS and LC-MS instrumentation are required to achieve the limits of quantitation. Although the limit of quantitation for many residues can be demonstrated by quantitating the MS1 parent ion and using MS/MS fragment ions for further confirmation, we show that using parallel-reaction monitoring to quantitate residues using all MS/MS fragments increases the selectivity and overall confidence when establishing the limits of quantitation in multi-residue methods. We also show that matrix-matched standards are imperative for accurate assessment of residues in cannabis products. Finally, we show that some residues are temperature labile in the LC-MS source which suggests that multiple LC-MS methods may be required for if additional levels of sensitivity are required. Collectively, this work demonstrates an approach for multi-residue methodology in cannabis products that may be applicable to other commodities or environmental samples that have very low, or a broad range of residue limits.

39 - Characterisation of the epigenetic reprogramming in perinatal male rat germ cells and its sensitivity to ethinylestradiol

Arlette Rwigemera[1], **Lisa-Marie Legault**[2]**, Serge McGraw**[2]**, Geraldine Delbes**[1]

[1]**INRS-Institut Armand-Frappier,** [2]**Université de Montréal - Centre de Recherche du CHU Sainte-Justine**

Epigenetic reprogramming is a key event of perinatal germ cells' development. Indeed, it guarantees genomic imprinting and cell differentiation. However, the kinetics and actors of this reprogramming are poorly characterized in rats. Moreover, it could be targeted by endocrine disruptors inducing long-term fertility disorders. Our study aims to 1- characterize the epigenetic dynamics in rat gonocytes during perinatal development; 2- test if this reprogramming is disturbed by exposure to a xenoestrogen. Using transgenic rats expressing GFP specifically in germ cells, we purified gonocytes by FACS at various stages of perinatal development and established the transcriptomic profile of 165 chromatin remodeling enzymes. In parallel, we determined the dynamics of DNA methylation (5mC) and six histone modifications by immunofluorescence on testes sections. Our results highlight a transient chromatin remodeling involving histone modifications during DNA remethylation. In parallel, we studied the effect of exposure to ethinylestradiol (EE2) used as a model compound of xenoestrogens since it is known to act on the estrogen receptor. We tested the impact of a 3 days exposure at 1μM, during DNA remethylation using the rat fetal testes culture model that we previously demonstrated reproduces epigenetic reprogramming. Our data showed that EE2 did not affect the number of gonocytes. Using DNA and RNA extracted from purified GFP-positive germ cells after exposure, we further tested the effect on DNA methylation and gene expression by RRBS (Reduced Representation Bisulfite Sequencing) and gene chip respectively. While analysis of the methylome is ongoing, we showed that expression of none of the enzymes studied above was affected. However, our data revealed that genes associated with olfactory transduction, mRNA translation, and cell adhesion were significantly affected. Our study established the kinetics of epigenetic reprogramming in male rat gonocytes and suggested that xenoestrogens may affect gene expression in these cells, independently of chromatin remodeling dynamics.

40 - Sub-acute and -chronic toxicological responses in male and female F344 rats following repeated oral dietary exposure to the processing-induced food contaminant 2-monochloro-1,3-propanediol

Jayadev Raju[1], **Jennifer. Roberts**[1], **Marnie Taylor**[1], **Don Caldwell**[1], **Tony Zhao**[1], **Dominique Patry**[1], **Jason Fine**[2], **Ivan Curran**[1], **Rekha Mehta**[1]

[1]_Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada.,_ [2]_Natural and Non-Prescription Health Products Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada._

2-monochloro-1,3-propanediol (2-MCPD) fatty acid esters are processing-induced food contaminants, occur in foods that contain refined vegetable oils (including infant formula), and can be rapidly hydrolyzed _in vivo_ to the parent compound. In addressing a regulatory toxicological data gap to understand the potential hazard of 2-MCPD, two independent animal studies were conducted according to the Organization of Economic Cooperation and Development Test Guidelines-407 and -408. Weanling male and female F344 rats (n = 10-12 rats/group/sex) were fed _ad libitum_ AIN-93G diets containing 2-MCPD to provide estimated daily doses of (a) 0 (control), 25, 50, 100 or 200 mg/kg BW for 28 days, and (b) 0 (control), 0.5, 2, 10 or 40 mg/kg BW for 90 days. Apical endpoints including tissue weights, histopathology, clinical serum biochemistry and hematology were evaluated. Residue analyses of the diets from both studies confirmed the intended concentration of 2-MCPD. In the 28-days study, given the moribund status that occurred within < 1 wk exposure span, the 100 and 200 mg/kg BW dose groups of 2-MCPD were excluded from the study. In both 28-day and 90-day studies, in comparison to the respective controls, non-neoplastic lesions (with interstitial vacuolation and mononuclear cell infiltration leading to focal inflammation, with fibrosis and necrosis) were observed in the heart tissues of rats exposed to 40 or 50 mg/kg BW 2-MCPD, respectively, together with significantly higher wet weights. Although relative wet weights of kidneys were increased in the 2-MCPD treated groups, no dose-specific changes in kidney pathology were found. For pathology lesions, we identify a no-observed effect level (NOEL) of 25 and 10 mg/kg BW 2-MCPD in the 28 and 90 days studies, respectively. While there were changes in a few parameters in the battery of tests conducted for clinical biochemistry and hematology, the differences were within the reference range. These detailed standardized sub-acute and -chronic dietary exposure studies consistently identify heart as a target organ for food-borne 2-MPCD in both male and female rats. Our data provides information to support the hazard identification of the processing-induced food contaminant 2-MCPD for regulatory purposes.

41 - A population-based study to explore the association between environmental contaminants and relevant cancers in Newfoundland and Labrador

Md Arifur Rahman[1], **Atanu Sarkar**[1], **Farah McCrate**[2]

[1]**Division of Community Health and Humanities, Faculty of Medicine, Memorial University of Newfoundland,** [2]**Eastern Health, NL**

Background: People in Newfoundland and Labrador (NL) are exposed to arsenic and Disinfection by-products (DBPs) by drinking contaminated water and pesticides used in the golf courses. Arsenic is a naturally occurring potent carcinogen found in ground water. DBPs are considered as possible human carcinogens and they are formed by chlorination of raw water containing organic substances. Several pesticides used in the golf courses are linked to certain types of cancers. However, there is no population-level spatial data on any association between the exposure to these contaminants and related cancers. The study aims to compare cancer prevalence rates between high and low exposure risk communities in NL.

Objective: An ecological study was conducted to explore any association between the exposure to arsenic and DBPs in drinking water and living close proximity to golf courses and prevalence of the relevant cancers at the community level of NL.

Methods: Based on the water quality data from provincial reports, the communities exposed to higher levels of arsenic and DBPs (Trihalomethanes and Halo-acetic acids) were identified. List of neighborhoods living within 500 meters of golf courses in NL were selected from google map. Communities with similar demographic characteristics except for the exposure to these contaminants were selected as low-risk groups. Literature search was done to make lists of cancers induced by arsenic, DBPs, and pesticides. Cancer data (histology and topography of cancers, sex, age, and postal code of the cases at the time of diagnosis) were extracted from the NL Cancer Registry for the cases diagnosed between 2007-2016. Relative risk and 95% confidence intervals (CIs) were calculated for statistical analysis.

Results: Communities with high arsenic, DBPs, and neighborhoods living within close proximity of golf courses had greater risk of developing certain cancers (RR 1.3, 95%CI, 1.03-1.51; RR 1.8, 95%CI 1.7-1.9; RR 1.8, 95%CI 1.5-2.0 respectively), than the low exposure communities. Males were at higher risk in both groups.

Conclusion: Population-level spatial distribution of environmental contaminants were significantly associated with higher risk of cancer. However, further studies are needed to explore genotoxicity to establish causal relationship.

42 - The Effects of BPA and its Analogues on Adipocyte Differentiation

Misha Singh[1,2], **A.Sorisky** [2,3], **Ella Atlas**[1,2]

[1]*Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada*,
[2]*University of Ottawa, Department of Biochemistry, Microbiology and Immunology*,
[3]*Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada*

Obesity and the metabolic disease associated with it are increasing in the Western population. A sedentary life style, poor diet and genetic predisposition do contribute to obesity; however, environmental chemicals such as Bisphenol A (BPA), may play a role. BPA has been correlated with an array of negative health effects thus forcing manufacturers to replace it with structural analogues such as Tetra Methyl Bisphenol F (TMBPF), Bisphenol F (BPF), Bisphenol AP (BPAP), and fluorine-9-bisphenol (BHPF). BPA is thought to be an obesogen due to its ability to induce adipogenesis in human and murine preadipocytes. The effects of the structural analogues of BPA, listed above, on adipogenesis have yet to be evaluated. Therefore, the focus of this study aims to investigate their adipogenic effects. For this purpose we used 3T3-L1 mouse embryonic fibroblasts. This cell model can be differentiated into mature adipocytes given appropriate inducers consisting of 3-isobutyl-1-methylxanthine (IBMX), insulin and dexamethasone, a synthetic steroid. To assess the effects of BPA analogues, the cells were treated with varying concentrations of TMBPF, BPF, BHPF, BPA, and BPAP, in place of dexamethasone. To assess the adipogenic potential of the chemicals the expression levels of the markers of mature adipocytes (aP2, Lpl, Perilipin, Pparγ, C/ebpα, Adiponectin, Adipsin) was assessed at mRNA and protein levels. Lipid accumulation was evaluated by Nile Red staining. TMBPF was found to be the most obesogenic and significanlty more potent than BPA at inducing some specific adipogenic markers. A time course was performed to assess the timely expression levels of known transcriptional regulators of adipogenesis. TMBPF upregulated key transcription factors C/ebpα and Pparγ as early as Day 2.

The results suggest that TMBPF is capable of inducing adipogenesis to a greater extent than the other chemicals including BPA. This was observed by increased expression of adipogenic markers and lipid accumulation. BHPF and BPAP did not affect adipogenesis in this model. Understanding the mechanisms and the potential role of chemicals in adipose tissue formation in vitro is vital to evaluating their potential link to obesity as well as other metabolic diseases.

43 - Study of the metal content of species of interest for fishing (demersal and pelagic) of the Galapagos Islands. Risk evaluation

Eduardo Franco Fuentes[1]**, Ángel José Gutiérrez Fernández**[1]**, Carmen Rubio Armendariz**[1]**, Dailos Glez Weller**[1]**, Arturo Hardisson de la Torre**[1]**, Jorge Ramírez González**[2]**, Solange Andrade Vera**[2]

[1]*1Universidad de La Laguna, Área de Toxicología, Tenerife, Islas Canarias, España.,*
[2]*2Fundación Charles Darwin, Puerto Ayora, Santa Cruz, Islas Galápagos, Ecuador.*

Introduction: Metal pollution in the marine environment can damage areas with exceptional biodiversity such as the Galapagos Islands. It is important to study whether there are differences in the metal concentrations of the demersal (*Caulolatilus princeps* and *Mycteroperca olfax*) and pelagic (*Thunnus albacares* and *Seriolella violacea*) species which are of commercial fishing interest, and evaluate the possible toxic risk from their intake.

Material and method: 87 individual specimens were sampled between February and May 2019 at the Pelikan Bay dock in the city of Puerto Ayora (Santa Cruz Island), with a minimum of 20 specimens (10 males and 10 females) of each species under study, except *M. olfax*, of which all the specimens obtained were female.

5 g of muscle, liver and gonadal tissue were obtained from each specimen, which were subsequently labeled and cold stored in the "Laboratory of the Interdisciplinary Project Fisheries of the Charles Darwin Foundation".The samples were sent to the Toxicology Area of the University of La Laguna, where the levels of Al, Cd, Pb and Ni were analyzed using ICP-OES.

The safety margins (MoS) and the estimated weekly intakes for Al, Cd, Pb and Ni were calculated using the tolerable weekly intakes set by the EFSA and a weekly intake of 230 g of fish muscle.

Results: Muscle tissue: *M. olfax*: 37.627±31.710 mg Al/kg and 2.543±10.341 mg Cd/kg fresh weight. *C. princeps*: 0.443±0.622 mg Pb/kg and 1.193±0.785 mg Ni/kg fresh weight.

Liver tissue: *C. princeps*: 39.367±30.246 mg Al/kg, 0.992±1.674 mg Pb/kg and 4.485±4.546 mg Ni/kg fresh weight. *S. violacea*: 71.539±31.290 mg Cd/kg fresh weight.

Gonadal tissue: *M. olfax*: 83.408±66.915 mg Al/kg and 1.597±3.094 mg Cd/kg fresh weight. *C. princeps*: 0.619±0.761 mg Pb/kg and 3.450±3.245 mg Ni/kg fresh weight.

Conclusions: Due to the high concentrations of Cd in the muscle tissue of *C. princeps* it is recommended not to consume more than 443 grams per week in the case of men and 380 grams per week in the case of women. *M. olfax* species could cause cadmium toxicity as it presents an MoS> 1, therefore no more than 86 and 73 grams of muscle should be ingested per week by men and women, respectively

44 - Trace elements and reproductive success of river otters (Lontra canadensis) in the Alberta Oil Sands Region

Robert Gutgesell[1], **Sergio Raez-Villanueva**[1], **Jonathan Cormier**[2], **Alison C. Holloway**[1], **Philippe J. Thomas**[3]

[1]**Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON,** [2]**Fish and Wildlife Branch, Energy and Resource Development, Government of New Brunswick, Fredericton, NB.,** [3]**Environment and Climate Change Canada, Ottawa, ON.**

Background: There is concern that bitumen extraction in the Alberta Oil sands region is leading to increased concentrations of trace elements (including heavy metals) in the environment. As there is clear evidence from laboratory studies that exposure to some of these compounds can adversely impact fertility in mammals, concerns have arisen regarding the impact of these elements on reproductive outcomes in wildlife species in the region. River otters (_Lontra canadensis_) are sensitive indicator species. Otters are being collected in the Alberta Oil Sands Region (AOSR) as part of the hunter-trapper harvested wildlife toxicology monitoring program. The goals of this study were 1) to evaluate reproductive success in river otters collected in the AOSR, and compare this to other populations in North America and 2) determine if metal residues differed between pregnant and non-pregnant animals

Methods: River otters are collected by Indigenous land users and registered trappers in the AOSR, including at downstream locations in the Peace Athabasca Delta. The carcasses are frozen and shipped to the National Wildlife Research Centre (Ottawa, ON). Reproductive success is determined by collection of blastocysts from the uterine tract (pre-implantation) or by counting the number of fetuses (post-implantation). Pregnancy rates (#females with blastocyst or fetus/total number of females) in river otters collected during the 2016-17 trapping season in Alberta (N=23) were compared with data collected from New Brunswick (1997-2017) and historical rates in North America identified through a literature review. Concentrations of 24 metals in liver samples were determined in a subset of otters (N=16) by inductively coupled plasma mass spectrometry (ICP-MS) after a nitric acid digestion of the samples.

Results: The pregnancy rate in otters captured in the AOSR in 2016-7 was 34.7%. This rate is lower than what was reported in the same year in New Brunswick (50%), and is below historical values reported in the literature (54-66%). Pregnant animals (N=6) had higher concentrations of cadmium ($P<0.05$) compared to the non-pregnant animals (N=10).

Conclusion: Although cadmium has been associated with impaired fertility, preliminary results are suggesting a positive relationship between increased Cd and pregnancy, a trend that will need to be confirmed by increased sample size and additional statistical modelling techniques. The pregnancy success rate in the AOSR appears to be lower than what has been reported elsewhere in North America, however, further monitoring is required to determine whether this is an isolated observation or if this trend is sustained over subsequent years.

45 - Naphthenic acids and metabolic health: a focus on ANGPTL4

Genevieve Perono[1], **Sergio Raez-Villanueva**[1], **Laiba Jamshed**[1], **Philippe J. Thomas**[2], **Alison C. Holloway**[1]

[1]*Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON.,*
[2]*Environment and Climate Change Canada, Ottawa, ON.*

Introduction: Oil sands processed-affected water (OSPW) are residual tailings water formed as a result of bitumen extraction from the Alberta oil sands. Naphthenic acids (NA) are a constituent of bitumen and OSPW. We previously demonstrated that NA exposure causes significant perturbations in peroxisome proliferator-activated receptor a (PPARα) mediated metabolic pathways, profoundly increasing expression of angiopoietin-like protein 4 (*Angptl4*), an important regulator of lipid homeostasis. Interestingly, *Angptl4* has also been shown to be regulated by glucocorticoid receptor (GR) mediated pathways as well. Since OSPW and NA have been reported to have activity at both PPARα and GR, the goal of this study was to understand transcriptional regulation of *Angptl4* as a result of NA exposure.

Methods: McA-RH7777 cells, a rat hepatoma cell line, were exposed to 1.25, 25, and 125 mg/L of a technical NA mixture for 24h and 48h at concentrations within the reported range of NA in OSPW. To determine whether NA effects are PPARα-mediated, we compared *Angptl4* expression to other known PPARα targets [cartinine palmitoyltransferase 1 (*Cpt1*); solute carrier family 25 member 20 (*Slc25a20*)] when exposed to NA or a selective PPARα agonist, WY-14643. Similarly, we compared the effects of NA and a selective GR agonist, dexamethasone, on the expression of a known GR target [tryptophan 2,3-dioxygenase (*Tdo2*)]. We used a PPARα-selective antagonist (GW6471) and a GR-selective (mifepristone) antagonist, to evaluate whether the NA induction of *Angptl4* could be blocked.

Results: NA treatment dose-dependently increased expression of *Angptl4* at 24h and 48h. NA treatment did not result in any changes in the GR target *Tdo2*, but it did significantly increase the expression of the PPARα targets *Cpt1* and *Slc25a20* expression. However, neither PPARα-selective nor GR-selective antagonists were able to completely prevent the NA-induced increases of *Angptl4* expression.

Conclusion: *Angptl4* is an important regulator of lipid metabolism in the liver that can be modulated by several molecular pathways. Although we previously reported that exposure to commercial technical NA increases direct targets of PPARα, results of this study show that NA exposure may be causing metabolic perturbations by alternative molecular pathways. As PPARα and PPARγ regulate many of the same genes, and NA may also act as a PPARγ ligand, future studies are needed to investigate whether changes in key hepatic metabolic targets are mediated via NA effects on PPARγ.

46 - Application of high-throughput transcriptomics in human liver spheroids to facilitate read-across data for risk assessment of 24 per- and polyfluoroalkyl substances (PFAS)

Anthony J.F. Reardon[1], **Andrea Rowan-Carroll**[1], **Stephen S. Ferguson**[2], **Remi Gagne**[1], **Byron Kuo**[1], **Karen Leingartner**[1], **Andrew Williams**[1], **Christine L. Lemieux**[3], **Luigi Lorusso**[4], **Christine Levicki**[4], **Julie A. Bourdon-Lacombe**[3], **Richard Carrier**[3], **Ivy Moffat**[3], **Carole L. Yauk**[1], **Ella Atlas**[1]

[1]_Environmental Health Science & Research Bureau, Healthy Environments & Consumer Safety Branch, Health Canada_**,** [2]_Biomolecular Screening Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences National Institutes of Health,_ [3]_Water & Air Quality Bureau, Healthy Environments & Consumer Safety Branch, Health Canada_**,** [4]_Chemicals & Environmental Health Management Bureau, Safe Environments Directorate, Healthy Environments & Consumer Safety Branch, Health Canada_

Per- and polyfluoroalkyl substances (PFAS) are some of the most prominent organic contaminants in human blood that have the potential to disrupt biological processes and pathways in the liver. Although the toxicological implications from human exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are well established, data on other, lesser-understood PFAS are limited. A major challenge for regulatory authorities is to establish acceptable levels of human exposure to large, diverse classes of environmental contaminants such as PFAS. New approach methodologies (NAMs) that apply bioinformatics tools to interpret biological data are being increasingly considered to inform risk assessment when traditional toxicology methodologies are not amenable. The aims of the current investigation were to identify the biological responses relevant to PFAS mode of action, determine point of departure (POD) concentrations for these responses, and integrate this information to inform/facilitate read-across for risk assessment of data-poor PFAS.

A TempO-Seq platform (BioSpyder) measured gene expression changes in human liver microtissues (i.e., spheroids) after 24hr exposure to increasing concentrations of PFAS, with concurrent cytotoxicity measures. A bioinformatic framework developed from examining 4 representative PFAS (i.e., PFOS, PFOA, PFBS (short-chain) and PFDS (long-chain)) was applied to the broader PFAS group (24 total), sub-classed as carboxylates (PFCAs), sulfonates (PFSAs), or precursors. Subclasses were analyzed for total number of altered transcripts, identifying target genes of interest and exploring corresponding biological response pathways affected by PFAS exposures. The PODs for each PFAS were determined using freely-available benchmark concentration software (BMDExpress v2.2).

Both PFCAs and PFSAs exhibited a trend toward increased transcriptional changes with carbon chain-length. Specifically, longer-chain compounds (7 to 10 carbons) were more likely to surpass liver-toxic transcriptomic thresholds established from previous studies than shorter chain PFAS. Longer-chain PFAS were also more potent, inducing transcriptional effects at lower concentrations; however, PFOS was the most potent PFAS and of all precursors, only PFOSA (a PFOS precursor) induced a response. The combined high-throughput transcriptomic and bioinformatics analyses revealed the capability of NAMs to assess the effects of PFAS in liver microtissues; such data improves our understanding of PFAS-related effects in humans and facilitates the use of read-across in human health risk assessment for other data-poor chemicals.

47 - Examining the predictors of circulating arsenic levels in the Canadian population

Katherine Pullella[1,2], Shelley A. Harris[3,4], Anthony J. Hanley[1,3], Steven A. Narod[1,2,3], Joanne Kotsopoulos[1,2,3]

[1] Department of Nutritional Sciences, University of Toronto
[2] Women's College Research Institute, Women's College Hospital
[3] Dalla Lana School of Public Health, University of Toronto
[4] Occupational Cancer Research Centre, Cancer Care Ontario, Toronto

Introduction: Arsenic is an established carcinogen for cancers of the bladder, skin and lungs; yet its relationship to other cancers, including breast cancer, remains unclear. Our team recently reported a significant, linear relationship between total blood arsenic levels and breast cancer (hazard ratio [HR] quartile 4 vs. 1 = 13.2, 95%CI 4.02-43.0 *P*-trend = <0.0001), suggesting arsenic is a risk factor even in a low-exposure population. Consumption of contaminated water, rice, and fish products are established predictors of arsenic status; however, this is based primarily on studies conducted in high-exposure populations. Determining predictors of arsenic in Canada, a low-exposure population, is necessary to reduce the burden of cancer from exposure to an environmental carcinogen. Thus, the purpose of this study is to (1) identify the key predictors of circulating arsenic levels, and (2) to evaluate the relationship between circulating arsenic levels and cancer risk in a Canadian population.

Methods: Exposure data (e.g. diet, water), demographic information, and blood arsenic levels from the Canadian Health Measures Survey (CHMS) dataset will be utilized. The CHMS is an ongoing, nationally representative survey, collecting objective health measures and food intake information from participants in biennial cycles. Linkage to the Canadian Cancer Registry (CCR) will be performed to determine incident cancers (total and site specific). Logistic regression will be used to identify predictors of blood arsenic and Cox proportional hazards will be used to estimate the relationship between arsenic status and cancer risk (total and site specific). Linkage to CCR and data analysis is ongoing.

Significance: This study will identify key sources of arsenic within the Canadian population and provide evidence supporting arsenic as an environmental carcinogen for breast and/or other cancers. This research is essential to develop crucial public health interventions needed to reduce cancer incidence in Canada.

48 - Impact of anthocyanin-rich meals on polychlorinated biphenyl-induced intestinal dysbiosis, oxidative stress and inflammation

Fang Lu[1], **Alexandra Kerasias**[1], **Michèle Iskandar**[1], **Stan Kubow**[1]

[1]*School of Human Nutrition, McGill University, 21111 Lakeshore, Ste. Anne de Bellevue, Montréal, QC H9X3V9, Canada.*

Background: Population studies have indicated increased risk for chronic inflammatory diseases from excessive consumption of foods containing a higher content of dioxin and non-dioxin-like polychlorinated biphenyls (PCBs) such as PCB126 and PCB153 congeners, respectively. A human population study indicated that dietary PCBs lead to a diminished microbial diversity associated with higher fecal concentrations of short-chain fatty acids (SCFAs), as noted in subjects with cardiometabolic disorders. PCBs could disrupt gut microbial community structure leading to opportunistic pathogens producing endotoxins that can cause mitochondrial dysfunction and proinflammatory mitochondrial DNA fragments. PCB toxicity could be counteracted by diets rich in anthocyanins (ANs) with prebiotic features to support gut microbial health in addition to antioxidant and anti-inflammatory properties associated with microbial metabolites of ANs.

Methods: An *ex vivo* human simulated gut model involving human fecal microbiota was used to digest AN-rich potato meals in the presence and absence of PCBs. Fecal water (FW) digests were collected for antioxidant power, SCFA profiles and gut microbial profiles (16s) analysis. T84 human colonic cells were used to assess whether digests from AN-rich meals could protect against the cytotoxicity induced by PCB containing digests. Cells were harvested for assessment of viability (MTT), superoxide (MitoSOX), mitochondrial function (Mitoplate) and inflammasome NLRP3 (western blotting and ELISA). Cell culture supernatants were collected for cytokine and chemokine analysis (Luminex).

Results: AN-containing fecal water digests in the presence of PCBs showed significantly (P<0.05) higher antioxidant capacity as compared to PCBs digests only. The digests containing PCBs and ANs showed significantly (P<0.05) higher T84 cell viability as compared to PCBs digests at fecal water concentrations of 1% and 5%. PCBs digests were associated with a tendency for increased individual SCFAs including acetate, butyrate and propionate that was lowered in the presence of AN-containing digests.

Conclusions: AN-rich potato meal digests are protective against PCB digest-induced cytotoxicity, which may be partly mediated via antioxidant effects. Ongoing investigations include specifying the gut microbial profiles, analyzing mitochondrial functions and the activation of TLR5 receptors and qualifying and quantifying the inflammasome and downstream cytokines and chemokines.

49 - Development of DNA methylation based in vitro assay for the identification of carcinogens

Daniel Desaulniers[1], **Cathy Cummings-Lorbetskie[1]**, **Rémi Gagné[1]**, **Karen Leingartner[1]**, **Gong-Hua Xiao[1]**, **Ruth Wilkins[2]**, **Jessica C Pickles[3]**, **Carole L Yauk[1]**, **Robert F Newbold[4]**

[1]_Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada, K1A 0K9_, [2]_Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada, K1A 1C1_, [3]_University College London Great Ormond Street Institute of Child Health, London, UK, WC1N 1EH_, [4]_Brunel University London, Uxbridge, Middlesex, UB8 3PH, UK_

Regulatory agencies rely on rodent cancer bioassays to identify carcinogenic chemicals, but such bioassays are not frequently conducted and will be phased out by the US-EPA. Some short-term assays can detect genotoxic carcinogens but no validated method exists to detect non-genotoxic carcinogens, i.e. those lacking inherent DNA damaging properties. Epigenomic anomalies that are predictive of early events in carcinogenesis may provide new and complementary cancer bioassays. Our goal is to find early epigenetic markers that inform carcinogenic modes of action. The objective is to establish the chronology of DNA methylation changes from primary cells through senescence bypass, and identify DNA methylation marks following carcinogen exposure that enable entry into the early oncogenic stages of senescence-bypass and immune-evasion. We hypothesize that such epigenetic changes will be informative carcinogenesis biomarkers. The Syrian hamster fetal cell model was selected because: a) it has been used in colony transformation assays to detect chemical carcinogens over the past 50 years, b) the cells have normal karyotype and an intact DNA repair system, c) it is more similar to humans than rat or mouse cells (metabolically and spontaneous transformation rate). Following a 7-day _in vitro_ exposure to the carcinogen benzo[a]pyrene, cells were collected through time until after senescence bypass. DNA methylation was investigated using pyrosequencing, reduced representation bisulfite sequencing (RRBS), and a global genome luminometric assay. Preliminary results support colony dependent changes in DNA methylation as they reached and escaped senescence. A decrease in methylation occured after 27 days in retrotransposons (Line-1-like) as the cells started to senesce and the time required to divide increased from 1 to 2.5 days. RRBS analyses showed a persistent 30% drop in global genome DNA methylation in some, but not other colony-derived cell lines. Gene-specific methylation is currently being investigated. Activation of Line-1 occurs during senescence in human and contributes to cancer. Here, Line-1 becomes demethylated as the cells reach senescence providing a possible mechanism for its activation. Overall, these results suggest that epigenetic anomalies may serve as early indicators of cancer predisposition. Funded by Chemicals Management Plan, Health Canada.

50 - Development and application of an analytical pipeline for high-throughput gene expression profiling of per- and poly-fluoroalkyl substances (PFAS) in primary liver human spheroids to inform read-across

Andrea Rowan-Carroll[1], **Karen Leingartner**[1], **Remi Gagne**[1], **Anthony Reardon**[1], **Byron Kuo**[1], **Andrew Williams**[1], **Julie Bourdon-Lacombe**[2], **Ivy Moffat**[2], **Richard Carrier**[2], **Stephen Ferguson**[3], **Luigi Lorusso**[4], **Christine Levicki**[4], **Ella Atlas**[1], **Carole Yauk**[1]

[1]*Healthy Environments and Consumer Safety Branch, Environmental Health Science and Research Bureau, Health Canada,* [2]*Healthy Environments and Consumer Safety Branch, Safe Environment Directorate, Water and Air quality Bureau, Health Canada,* [3]*Biomolecular Screening Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences,* [4]*Healthy Environments & Consumer Safety Branch, Safe Environments Directorate, Chemicals and Environmental Health Management Bureau, Health Canada*

Per- and poly-fluoroalkyl substances (PFAS) are widely found in the environment due to their extensive use, persistence, and high mobility. Exposure to PFAS is an international concern, due to their potential to cause liver toxicity and reproductive/developmental effects. There is a body of knowledge on the toxicity of PFOS (perfluorooctane sulfonic acid) and PFOA (perfluorooctanoic acid); however, few data exist for many of the other PFAS.

A non-subjective analytical strategy is required to assess PFAS that lack conventional toxicity testing data. To this end, an approach was developed to screen for PFAS-induced biological perturbations through global gene expression profiling. This pilot study focusses on four model PFAS to establish the methodologies and approach: PFOS, PFOA, perfluorobutanesulfonic acid (PFBS), and perfluorodecanesulfonic acid (PFDS). Human primary liver cell spheroids were exposed to 10 concentrations of PFAS and examined at four time-points. Targeted RNA-sequencing was used to identify changes in gene expression profiles, and a bioinformatic pipeline was developed.

This approach aims to: (1) identify biological responses relevant to PFAS mode of action; (2) determine at what concentrations these substances exert their toxic effects; (3) determine how to harness these data/tools for read-across.

Results from the analytical pipeline determined; how many genes were altered by each PFAS, the prevailing biological changes, and the concentration at which each PFAS altered cell biology. Applying the pipeline revealed that all four PFAS induced robust changes in gene expression. PFBS was found to be the least biologically active and least potent, while PFOS was found to be the most potent across all time points. Interestingly both PFOS and PFDS were found to have very similar expression profiles over all time points.

The use of high-throughput gene expression profiling and the developed analytical pipeline can be used to compare and contrast biological activities and potencies to inform read-across. Results from this study indicate that while PFAS may work through a variety of transcription factors some common modes of action do exist. Data generated through this research will be used to inform future risk assessment of PFAS.

51 - Synthesis and Characterization of a Novel C8, N7-Deoxyguanosine Cyclic Adduct

Trevor W. Manning[1], **Richard A. Manderville**[1], **P. David Josephy**[2], **Ryan W. Kung**[3], **Stacey D. Wetmore**[3]

[1]**Departments of Chemistry & Toxicology, University of Guelph, Guelph, ON, Canada N1G 2W1**, [2]**Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada N1G 2W1**, [3]**Department of Chemistry & Biochemistry, University of Lethbridge, Lethbridge, AB, Canada T1K 3M4**

Nitroaromatic compounds represent a major class of industrial chemicals that are also found in nature. Evidence indicates that nitroaromatics are metabolized into reactive, electrophilic nitrenium ions that can react with DNA bases. Specifically, the reaction of the nitrenium ion with the C8 position of 2'-deoxyguanosine produces characteristic N-linked adducts that facilitate mutagenesis and carcinogenesis. Recently, it has been reported that cyano substitution can greatly increase the mutagenicity of nitroaniline derivatives. The basis of this "cyano effect" may be rooted in the formation of a novel cyclic C8, N7-2'-deoxyguanosine adduct that is structurally characterized in this poster. Density functional theory (DFT) calculations, molecular dynamics (MD) simulations and NMR analysis indicate that this emissive adduct adopts the *syn* conformation and can stabilize the slipped mutagenic intermediate (SMI), providing a rationale for the potent mutagenicity observed in frameshift-sensitive tester strains

52 - Variability in stress axis function alters ozone-induced lung immune cell responses

Jith Thomas[1], **Dalibor Breznan**[1], **Andrew Stalker**[2], **Josée Guénette**[1], **Errol Thomson**[1]

[1]*Environmental Health Science and Research Bureau, Health Canada, Ottawa, Canada, K1A 0K9,* [2]*Biologics and Genetic Therapies Directorate, Centre for Biologics Evaluation, Health Canada, Ottawa, Canada, K1A 0K9*

Exposure to ozone, a criteria air pollutant, has been linked to respiratory morbidity and mortality. As a highly reactive gas, ozone is entirely consumed in the lungs, and it has been suggested that most respiratory adverse effects of this pollutant are mediated by the release of bioactive secondary mediators and/or modification of lung immune cell response. We have previously demonstrated that ozone inhalation activates the hypothalamic-pituitary-adrenal (HPA)/stress axis, resulting in increased levels of stress hormone (corticosterone in rodents and cortisol in humans) in the circulation and lungs. Macrophages are the prominent immune cells present in the lungs of healthy individuals. Here we studied whether differences in stress hormone production modify inflammatory responses in lung macrophages collected from ozone exposed rats and further verified the role of stress hormone in modifying the pulmonary macrophage function in a human monocyte-derived macrophage cell line (THP-1). Two rat strains with known differences in their stress axis function, highly-stress responsive Fischer and less responsive Lewis, were exposed by nose-only inhalation to air or ozone (0.8 ppm) for 4 hours. The hyper-stress responsive Fischer rats had higher corticosterone levels in the lung milieu when compared to the hypo-stress responsive Lewis rats before and after ozone exposure. Lung macrophages collected from rat strains after ozone exposure showed distinct pro-inflammatory gene expression patterns. An enhanced pro-inflammatory response was observed in the hypo-stress responsive Lewis rat when compared to the hyper-stress responsive Fischer rats suggesting that stress hormone is a potential modifying factor in ozone-induced lung inflammatory responses. Furthermore, in vitro incubation of THP-1 macrophages with stress hormone modified pro-inflammatory gene expression and the phagocytic response of the cells. Overall, our results suggest that stress hormone is a determining factor in regulating lung immune cell responses to ozone.

53 - In Vitro Determination of Drug Transporter Interactions Using LC-MS/MS Quantitation

Albert Licollari[1], **Andy Quaile[1], Richard Bagshaw[1], Gordon Bolger[1]**

[1]**_Nucro-Technics_**

Interactions with drug transporters play a critical role in the ADME and toxicity of drugs. _In vitro_ cell systems that express transporters are available to study these interactions. More commonly, selective radiolabeled or fluorescent substrates are employed to evaluate the interaction of drug candidates at transporters. For direct measurement of drug transport in cell systems, not all candidate drugs may possess intrinsic fluorescence and the production of a radiolabeled candidate drug can be challenging. Therefore, we have examined the use of LC-MS/MS quantitation as an alternative to characterize drug transporter interactions employing Solvo's PREDICELL[TM] monolayer cell cultures in a 24-well format expressing human OATP1B1, OAT3, OCT2 and MATE1 transporters and PREADYPORT[TM] 24-transwell permeability assay format expressing P-gp. The following selective transporter substrates and inhibitors were chosen for this study: OATP1B1 (estradiol-ß-glucuronide 5 µM and cyclosporine 10 µM), OAT3 (estrone sulfate 10 µM and diclofenac 25 µM), OCT2 (metformin 1000 µM and verapamil 30 µM), MATE1 (tetraethylammonium 30 µM and cimetidine 10 µM) and P-gp (digoxin 5 µM, an efflux substrate and cyclosporine 10 µM, an inhibitor of efflux). Transporter substrates or substrates plus inhibitor were incubated with monolayer cell cultures for 20 min at 37ºC. Uptake and the efflux of digoxin, and efflux of digoxin in the presence of inhibitor were measured for 2 hrs at 37ºC. Uptake of substrates in monolayer cells was measured following extraction into 50% methanol:water, while for the permeability measurements, substrates were extracted using a solid-phase extraction method. Concentrations of substrates were measured by LC-MS/MS. For OATP1B1, OAT3, OCT2 and MATE1 transporters, large differences of transporter substrate uptake of 10, 19, 230 and 9-fold were observed between transporter expressing and non-expressing cells, respectively, with selective inhibitors of transport inhibiting by 71%, 88%, 99% and 15%, respectively. Digoxin displayed 35-fold greater efflux compared to uptake, and the efflux of digoxin was inhibited 30% by cyclosporine. These observations demonstrate that our LC-MS/MS methods are well suited to assessing drugs as potential substrates or inhibitors of human expressed transporters.

54 - Cytotoxicity screening and ultrastructural study of nonporous silica nanoparticles uptake by mammalian cells

Dalibor Breznan[1], **Nazila Nazemof**[2], **James Gomes**[2], **Prem Kumarathasan**[1]

[1]*Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON*, [2]*Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON*

Silica nanoparticles (SiNPs) have found widespread use in biomedical applications such as bioimaging, dentistry, orthopedics, as well as manufacture of sensors, semiconductors, ceramics and construction materials. Their large-scale production and use based on their attractive physicochemical properties related to their nano-size (e.g. large surface area, relative biocompatibility and potential degradability, optical transparency, ordered structure) increases the likelihood for environmental and human exposure. Therefore studies are ongoing to examine the safety of SiNPs and to assess the risks from exposures to these nanomaterials. We have previously studied the safety profiles of mesoporous silica nanoparticles. In the present work we studied a newly synthesized panel of nonporous SiNPs with varied size (15, 30, 50, 75 and 100 nm) and surface properties (C3-COOH, C11-COOH, $-NH_2$ and -PEG). We examined the cytotoxicity of this SiNP panel in J774 mouse macrophages and A549 human lung epithelial cells by profiling the cells for cell membrane damage (release of cytosolic lactate dehydrogenase, LDH), metabolic activity (resazurin reduction) and energy metabolism (ATP production). Also, mouse macrophages exposed to the SiNP panel were subjected to Transmission Electron Microscopic (TEM) analysis to examine the intracellular localization of the SiNPs after the 24-h exposure period. The study revealed that both cell lines were impacted by the particle exposures across the 10-100 $\mu g/cm^2$ dose range examined. The majority of the SiNPs showed low cytotoxic potency. These were either >50 nm in size and/or C3-COOH, or PEG-modified. Higher cytotoxic potency was observed for SiNPs <50 nm particles and/or pristine, NH_2 or C11-COOH-modified (e.g. LDH release). For most SiNPs, localization in cytoplasmic vesicles, large vacuoles and autophagosomes was observed within the macrophages. In contrast, pristine 15 nm-sized and PEG-modified 30 nm-sized SINPs were spread-out in large numbers across cytoplasm and within the pseudopodia of the exposed macrophages, in addition to their localization in large vacuoles. On some occasions, SiNPs were present in the vicinity of organelles including nucleus, mitochondria and endoplasmic reticulum. Additional analyses will be required to determine the mechanistic and functional implications of the differentially localized SiNPs in the cells. The work is aimed to address data gaps to enable more effective, evidence-based risk (hazard and exposure) assessment of the SiNPs.

55 - Nano silica exposure and protein changes in mitochondria from J774 macrophage cells

Prem Kumarathasan[1], Nazila Nazemof[2], Dalibor Breznan[1], Erica Blais[1], James Gomes[2], Mohan Babu[3], Renaud Vincent[1]

[1]Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON, [2]Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, [3]Department of Biochemistry, University of Regina, Regina, SK

Silica nanoparticles (SiNPs), due to their attractive physicochemical properties (e.g. size, surface area, electronic properties) have wide range of applications in consumer products, engineering and medical technologies. Thus, enhanced potential for exposure to SiNPs with small size and increased surface area also raises health concerns. Risk assessment of SiNP exposures requires toxicity information on these materials, which is currently a knowledge gap and especially, mechanistic understanding of SiNP toxicity is yet to be addressed. We have observed internalization of SiNPs in mouse macrophages and their localization on mitochondrial membrane and within this organelle, by electron microscopy analyses. In this work, we exposed mitochondrial fraction from J774 macrophage cells to custom-synthesized well-characterized amorphous pristine (15, 30, 75 nm) and surface-modified (-C3-COOH, -C11-COOH, -$(OH)_2Si(OCH_2CH_2)nOCH_3$ (n = 9-12) (-PEG) and -$(OH)_2Si(CH_2)_2CH_2NH_2$ (-NH_2)) SiNPs (15 nm), and assessed protein changes by mass spectrometry, to explore toxicity mechanisms. Our results showed SiNP size- and surface modification-related changes in various mitochondrial proteins (n=200) at the two exposure doses tested (5 and 15µg/cm2). However, 32 of these mitochondrial protein changes were detectable across all particle exposures. Some of these changes were related to respiratory complex proteins (e.g. cytochrome c oxidase, ATP synthase subunit, electron transfer flavoprotein subunit) and oxidative stress (e.g superoxide dismutase). Furthermore, SiNP exposure dose-related changes were also seen in these protein responses. Our findings from this relative toxicity screening approach reveal that size of and surface functionality on the SiNPs can potentially affect mitochondrial functions upon exposure and demonstrate the use of this approach in gaining valuable mechanistic information on SiNP toxicity *in vitro*.

56 - Docosahexaenoic acid (DHA) derived oxylipins are decreased in the heart by dietary exposure to 2-monochloro-1,3-propanediol

Lucien Cayer[1,2], Jennifer Roberts[3], Don Caldwell[3], Jayadev Raju[3], Harold Aukema[1,2]

[1]Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada, [2]Canadian Centre for Agri-Food Research in Health and Medicine, St Boniface Hospital Research Centre, Winnipeg, MB, Canada, [3]Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Canada.

Chloropropanols (CP) have been identified as chemical process-induced food contaminants, which occur as by-products of the manufacturing of refined food oils and hydrolyzed vegetable protein. The 3-monochloro-1,2-propanediol (-MCPD) isomer is the most abundant and well-studied of the CP and is a known rodent and 'possible human' (IARC Group 2B) carcinogen, with an established tolerable daily intake (TDI). Estimated high intakes for adults do not exceed the 3-MCPD TDI, however, intake estimates for infants exclusively formula-fed do exceed the TDI. There has been a paucity of research in understanding the hazard of the 2-MCPD isomer, thus forming a regulatory data gap for risk assessment. Health Canada conducted a 90-days sub-chronic dietary exposure study in F344 rats and identified 2-MCPD as a cardiotoxin, while skeletal muscle was unaffected (unpublished observation). Oxylipins are oxygenated metabolites of polyunsaturated fatty acids that can mediate cellular processes such as apoptosis, inflammation, and cell proliferation; and are emerging as potential biomarkers of toxicity. The main objective of this study was to understand the relationship between dietary 2-MCPD exposure and the oxylipin profile in rat heart and skeletal muscle to determine potential modes of action in these tissues. We conducted oxylipin analyses using HPLC-MS/MS in the hearts and skeletal muscles of male and female rats exposed to control and 2-MCPD (40 mg/kg BW) AIN-93G formulated diets for 90-days (Health Canada study). By comparison to the control, 5 of the 6 docosahexaenoic acid (DHA)-derived oxylipins were significantly lower in the 2-MCPD-treated hearts. In contrast, there were no alterations in oxylipin profiles between control and 2-MCPD-treated skeletal muscle. The DHA derived oxylipins are considered anti-inflammatory, and our results of their lower levels in the 2-MCPD-treated hearts suggests an inability to resolve marcrophage-induced inflammation and thus induce cardiotoxicity. This study provides a detailed profiling of oxylipins (in heart and skeletal muscle) and their potential roles as cardiotoxicity biomarkers of 2-MCPD and other chemical exposures.

57 - Evaluation of QSAR models for predicting fraction unbound in plasma in humans

Yejin Esther Yun MSc[1], Moriah Pellowe BSc[1], Andrea Edginton PhD[1]

[1]School of Pharmacy, University of Waterloo, Waterloo, Ontario

Introduction
When children are exposed to environmental toxicants, their immature anatomy and physiology can lead to higher blood concentration levels and a longer duration in the body as compared to adults. Physiologically-based toxicokinetic (PBTK) models can be used to simulate various exposure scenarios in children. Yun and Edginton [1] indicated that an accurate determination of a fraction unbound in plasma (fup) in children is necessary in PBTK modeling. When an observed fup in adults (fup_{adult}) of a compound is available, the precision of fup in children (fup_{child}) estimate will be dependent on the predictive performance of the ontogeny model (i.e. adult-to-child scaling model), which is a function of plasma protein concentrations at a specific age and fup_{adult}. When observed fup_{adult} data are not available, QSAR models can be used. The predictive accuracy of QSAR models is critical for predicting fup_{child}. Available QSAR models were developed based on training sets containing drug-like compounds. It is necessary, therefore, to compare the prediction accuracy of the QSAR models for environmentally relevant and pharmaceuticalcompounds. The objective is to evaluate the predictive performance of Watanabe et al. [2]and a commercial program, ADMETpredictor (Simulation Plus) for two classes of compounds.

Method
In order to assess the predictive performance of QSAR models, observed fup data were obtained from the literature. The input chemical structure files were obtained fromPubChem. QSAR-predicted fup was then compared to the observed. A precision metric such as median absolute error (MAE) was calculated.

Preliminary results
The test set included both pharmaceutical (n=478) and environmentally relevant compounds (n=718). ADMETpredictor resulted in slightly better prediction accuracy with MAE values of 0.11 (pharm) and 0.06 (tox) than Watanabe et al. with MAE values of 0.12(pharm) and 0.08 (tox). Both programs showed a comparable prediction accuracy for the compounds with low fup values (0.01 – 0.3), however, ADMETpredictor showed a higher prediction accuracy for compounds with high fup values (0.31-1).

Future direction
We will perform principal component analysis in order (i) to identify the most discriminating chemical characteristics between the two classes of compounds and (ii) to identify the chemical descriptors that contribute to low or high prediction error. The outcome of this study will elucidate an uncertainty associated with the use of QSAR models for predicting protein binding given physicochemical properties.

1. Journal of Toxicology and Environmental Health, Part A, 2019: p. 1-26
2. Mol Pharm, 2018. 15(11): p. 5302-5311.

58 - Direct comparison of Bisphenol A, Bisphenol F and Bisphenol S toxicities in a rat 28-day oral exposure study.

Guillaume Pelletier[1], Marc Rigden[1], Gen Sheng Wang[2], Bhaja Krushna Padhi[1] and Karen Leingartner[1]

[1]Hazard Identification Division, Environmental Health Science and Research Bureau, Health Canada

[2]Scientific Service Division, Health Product and Food Branch, Health Canada

Background: Bisphenol A (BPA) is present in a wide range of consumer products, but concerns about adverse human health effects have led to the emergence of replacement products such as Bisphenol F (BPF) and Bisphenol S (BPS). Studies assessing the in vivo toxicities of BPA, BPF and BPS can be difficult to compare, as they were performed in different animal models, using different exposure and experimental protocols. In order to better evaluate the relative in vivo toxicities of BPA, BPF and BPS, their effects were directly compared using a standardized regulatory toxicology exposure protocol.

Methods: Adult male Fischer rats were exposed by gavage for 28 consecutive days to BPA, BPF or BPS administered over a wide dose range. Rat health and dietary intakes were monitored throughout the exposure period, while urine and feces samples were collected. On the 29th day, rats were sacrificed under anaesthesia to collect blood and tissue samples.

Results and discussion: Preliminary data suggest that BPF and BPS are not inherently less toxic than BPA at high doses. BPS was the only bisphenol to significantly impair rat weight gains over the exposure period, but increased liver weights accompanied by perturbation of serum cholesterol levels were observed in both BPF and BPS-treated rats. Many BPA-treated rats presented mild signs of dehydration, despite significantly higher water consumption. However, relative kidney weights were significantly increased following exposure to all three bisphenols. The effects of BPA, BPF and BPS at lower doses on the conventional endpoints measured in standardized regulatory toxicology studies were very limited. Upcoming quantification of bisphenols and bisphenol metabolites in tissues and excreta, measurement of serum hormone levels and assessment of gene expression and histopathology in target organs should provide valuable information allowing for a comprehensive comparison of BPA, BPF and BPS relative toxicities at lower doses.

59 - Characterization of the metabolism of azo dyes by azoreductases from the human gut microbiome.

Riley Elder[1], David Josephy[1] and Emma Allen-Vercoe[1]

[1] University of Guelph, Canada

Azo dyes are the largest group of colorants produced and consumed worldwide. These dyes are used to improve the attractiveness of materials such as foods and pharmaceuticals. When ingested, the azo bonds are broken down by bacteria that have azoreductase enzymes within the gut. Many azo dyes have been removed from use in food and cosmetics due the toxicity and carcinogenicity of the metabolites. Tartrazine is a synthetic yellow azo dye commonly used as a food colourant; however, recent investigations report that tartrazine can induce immunotoxic and genotoxic effects. Tartrazine's metabolism and toxicity are not well understood. I hypothesize that the reduction of tartrazine by intestinal microbes can result in the production of metabolites that are detrimental to the human intestinal tract. My work will establish the metabolism of tartrazine through microorganisms found in the human gut and highlights the importance of understanding all possible metabolism products for food additives.

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